EVALUATION OF METABOLIC RISK FACTORS AND TOTAL ANTIOXIDANT CAPACITY IN TYPE 2 DIABETES MELLITUS IN URBAN POPULATION

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BONAFIDE CERTIFICATE

This is to certify that this dissertation entitled **"EVALUATION OF METABOLIC RISK FACTORS AND TOTAL ANTIOXIDANT CAPACITY IN TYPE 2 DIABETES MELLITUS IN URBAN POPULATION"** is the bonafide original work done by **Dr.B. SATHYA,** Post graduate in Biochemistry, under my overall supervision and guidance in the Department of Biochemistry, Govt. Kilpauk Medical College, Chennai, in partial fulfillment of the regulations of The Tamil Nadu Dr. M.G.R. Medical University for the award of **M.D Degree in Biochemistry (Branch XIII).**

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CERTIFICATE FROM THE GUIDE

This is to certify that the dissertation entitled **"EVALUATION OF METABOLIC RISK FACTORS AND TOTAL ANTIOXIDANT CAPACITY IN TYPE 2 DIABETES MELLITUS IN URBAN POPULATION"** is a bonafide research work done by **Dr. B. Sathya,** Post graduate in Biochemistry, under my guidance in the Department of Biochemistry, Govt. Kilpauk Medical College, Chennai, in partial fulfillment of the regulations of The Tamil Nadu Dr. M.G.R. Medical University for the award of **M.D Degree in Biochemistry (Branch XIII).** I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains pages from Introduction to Conclusion and the Result shows 3 percentage of plagiarism in the dissertation.

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ABBREVIATIONS

IDDM	-	Insulin Dependent Diabetes Mellitus
NIDDM	-	Non Insulin Dependent Diabetes Mellitus
T2DM	-	Type 2 Diabetes Mellitus
T1DM	-	Type 1 Diabetes Mellitus
GDM	-	Gestational Diabetes Mellitus
HDL	-	High Density Lipoprotein
LDL	-	Low Density Lipoprotein
DM	-	Diabetes Mellitus
BMI	-	Body Mass Index
HbA1C	-	Hemoglobin A_{1C}
FPG	-	Fasting Plasma Glucose
2h-PG- 2	-	Hours Post-prandial Glucose
MODY	-	Maturity Onset Diabetes of the Young
IGT	-	Impaired Glucose Tolerance
DNA	-	Deoxyribonucleic Acid
ATP	-	Adenosine Triphosphate
GLP-1	-	Glucagon Like Pepetide-1
IGF	-	Insulin-like Growth Factors
GLP	-	Glucagon Like Polypeptide
DKA	-	Diabetic KetoAcidosis
VEGF	-	Vascular Endothelial Growth Factor
TGF	-	Transforming Growth Factor

CVD	-	Cardio Vascular Disease
CAD	-	Coronary Artery Disease
PND	-	Peripheral Neural Disease
CKD	-	Chronic Kidney Disease
ESRD	-	End-Stage Renal Disease
AGE	-	Advanced Glycation End-products
CHF	-	Congestive Heart Failure
VLDL	-	Very Low Density Lipoprotein
Apo-B	-	Apolipoprotein B
IDL	-	Intermediate Density Lipoprotein
LSM	-	Lifestyle Modifications1
GIP	-	Gastric Inhibitory Pepetide

CONTENT

INTRODUCTION

Diabetes is a group of disorders defined by absolute or relative deficiency of Insulin leading to hyperglycemia. Among the first main causes of death Diabetes is one.[1] Type 2 Diabetes Mellitus is the most common form of diabetes and it accounts for 90- 95% of all diabetic cases.[2] Type 2 diabetes is rapidly growing into a potential epidemic in India. India ranked first in 2000, among countries having the largest population with type 2 Diabetes Mellitus. The prevalence of Diabetes in developing countries is about 70 million as predicted by World Health Organization (WHO)[3]. Human lifestyle and behavioural changes have dramatically increased the incidence of Diabetes worldwide.[4]The prevalence of Diabetes Mellitus would be doubled from 171 million (2000) to 366 million (2030), with India topping the list, as predicted by Wild et al.[5]

Inability of the beta cells to compensate for the reduced insulin action leads to beta cell dysfunction[6]. The involvement of pancreatic islets, the liver and the muscles were originally implicated in the pathogenesis of type 2 DM. This was called the "triumvirate theory". The current trend in the pathogenesis of diabetes is described as the "Ominous Octet" which involves dysfunction of eight organs leading to type 2 diabetes. The organs include the pancreatic β-cells, the muscles, the adipose tissues, the liver, the brain, the kidney, the pancreatic α-cells and the intestine[36]. Hyperglycemia in Diabetes generates free radicals [7,8] which stimulates NADPH oxidase, principally in

neutrophils.[9] Disruption of redox balance results in stimulation of cell-signalling pathways associated with inflammation, dysregulation of insulin signalling and development of diabetic complications.[10] Many biochemical pathways, including glucose auto-oxidation, polyol pathway, prostanoid biosynthesis and protein glycation which are associated with hyperglycemia have been implicated in the increased free radical-production in diabetic subjects[13,15 ,16].

Evidence of oxidative stress in diabetes has been provided either by the increased levels of degradative products of ROS[11-15,17], deficiencies of specific antioxidants[18] or reduction in total antioxidant capacity in diabetic patients[18]. However, relationships between the antioxidant status, glycemic control and the risk for development of chronic complications in individuals with diabetes are not completely clear.

Due to the cumulative effect of antioxidants, it is preferable to measure the holistic activity of all the antioxidants known as "total antioxidant capacity" (TAC) instead of analysing each and every antioxidant agent's activity[28,29]. Total antioxidant capacity (TAC) has been defined as the moles of oxidants that are neutralized by one litre of solution; it is a biomarker to determine the antioxidant potential of body fluids[30].Studies claim that there exists severe oxidative stress among patients with type 2 diabetes mellitus[31].

Oxidative stress, targets many forms of biomolecules and among them, lipids are the easiest. Lipid oxidation forms many secondary products and the most studied one is Malondialdehyde (MDA). This aldehyde is the most toxic and its interaction with DNA and lipids are potentially mutagenic and atherogenic[32]. There is considerable evidence that hyperglycemia, which is inevitable in diabetes, causes many of the major complications of diabetes including nephropathy, retinopathy, neuropathy. Hyperglycemia induces auto-oxidation of glucose resulting in the formation of free radicals beyond the scavenging abilities of the endogenous anti-oxidant system[33]. The resulting oxidative stress plays a key role in the pathogenesis of late diabetic complications.

Consuming anti-oxidants either naturally or via drugs has delayed the development of major vascular complications of diabetes and has delayed or even prevented the progress of Impaired glucose tolerance to frank T2DM. The antioxidants include polyphenols in fruits and vegetables, ascorbic acid in citrus fruits and alpha-tocopherol in nuts like almonds, walnuts. Some drugs that have additional antioxidant actions than their pharmacological actions include Gemfibrosil, a lipid-lowering fibrate. Anti-hyperlipidemic statins are also reported to possess antioxidant effects. Also it has been demonstrated that thiazolidinediones (oral anti-diabetic drug) exert their vaso-protective effect through their anti-oxidant capacity[34].

The risk factors to diabetes include hypertension, dyslipidemia, smoking, positive family history, insulin resistant conditions like PCOS, acanthosis nigricans. In epidemiological studies, reduced levels of HDL cholesterol are consistently associated with increased risk of type 2 diabetes [19,20]. Hence increasing plasma HDL cholesterol has been considered as a novel therapeutic option to reduce the risk of type 2 diabetes[21,22.23].Reduced HDL cholesterol and increased triglycerides are part of the diabetic dyslipidemia[24,25,26], high levels of triglycerides have recently been shown to be a marker of type 2 diabetes rather than playing a causal role[27]. The increased triglyceride level alters the cholesterol composition of HDL. This leads to increased clearance of HDL resulting in reduced HDL levels in blood, which again is an indirect marker of insulin resistance[37]. The characteristic dyslipidemia of type 2 diabetes includes high triglyceride levels, low HDL cholesterol levels with normal LDL cholesterol levels[24,25,26].

Recent approaches to prevent diabetes as well as alleviate diabetic complications include lifestyle modifications by improving glucose tolerance[35]. 30-60 minutes of aerobic exercise 3-4 times a week is recommended by the American Heart Association to prevent cardiovascular risk[38]. Another study showed that blood lipoprotein composition is affected by eating habits too[39]. So, a planned lifestyle modification includes improving physical activity and modifying dietary habits by avoiding high-calorie food and consuming more fibre and protein-rich meal. This could drastically reduce the burden of mortality and morbidity associated with Type 2DM. Atherogenic

index of plasma is a well-established marker for atherogenesis and its strong predictive value is proved by many prospective studies[40]. So, we decided to study and evaluate the metabolic risks by calculating the atherogenic index and to evaluate the total antioxidant capacity in type 2 diabetic patients.

REVIEW OF LITERATURE

Diabetes Mellitus (DM) is a metabolic syndrome with multiple etiological factors including hereditary and ecological factors leading to hyperglycemia[41]. Defective secretion of Insulin or its action or a mixture of both could result in hyperglycemia. It is a chronic health problem with a multitude of catastrophic yet preventable complications[42,43].

PREVALENCE:

Diabetes Mellitus (DM) is probably one of the oldest diseases known to man. It was first reported in Egyptian manuscript about 3000 years ago [44]. In 1936, the distinction between type 1 and type 2 DM was clearly made [45]. Type 2 DM was first described as a component of metabolic syndrome in 1988 [46]. Type 2 DM (formerly known as non-insulin dependent DM) is the most common form of DM characterized by hyperglycemia, insulin resistance, and relative insulin deficiency[47].Type 2 DM results from interaction between genetic**,** environmental and behavioural risk factors [48,49].

RISK FACTORS[175]

Family history of diabetes (i.e., parent or sibling with type 2 diabetes)

Obesity (BMI ≥25 kg/m2 or ethnically relevant definition for overweight)

Physical inactivity

Race/ethnicity (e.g., African American, Latino, Native American, Asian American, Pacific Islander)

Previously identified with IFG, IGT, or an hemoglobin A1c (HbA_{1C}) of 5.7–6.4%

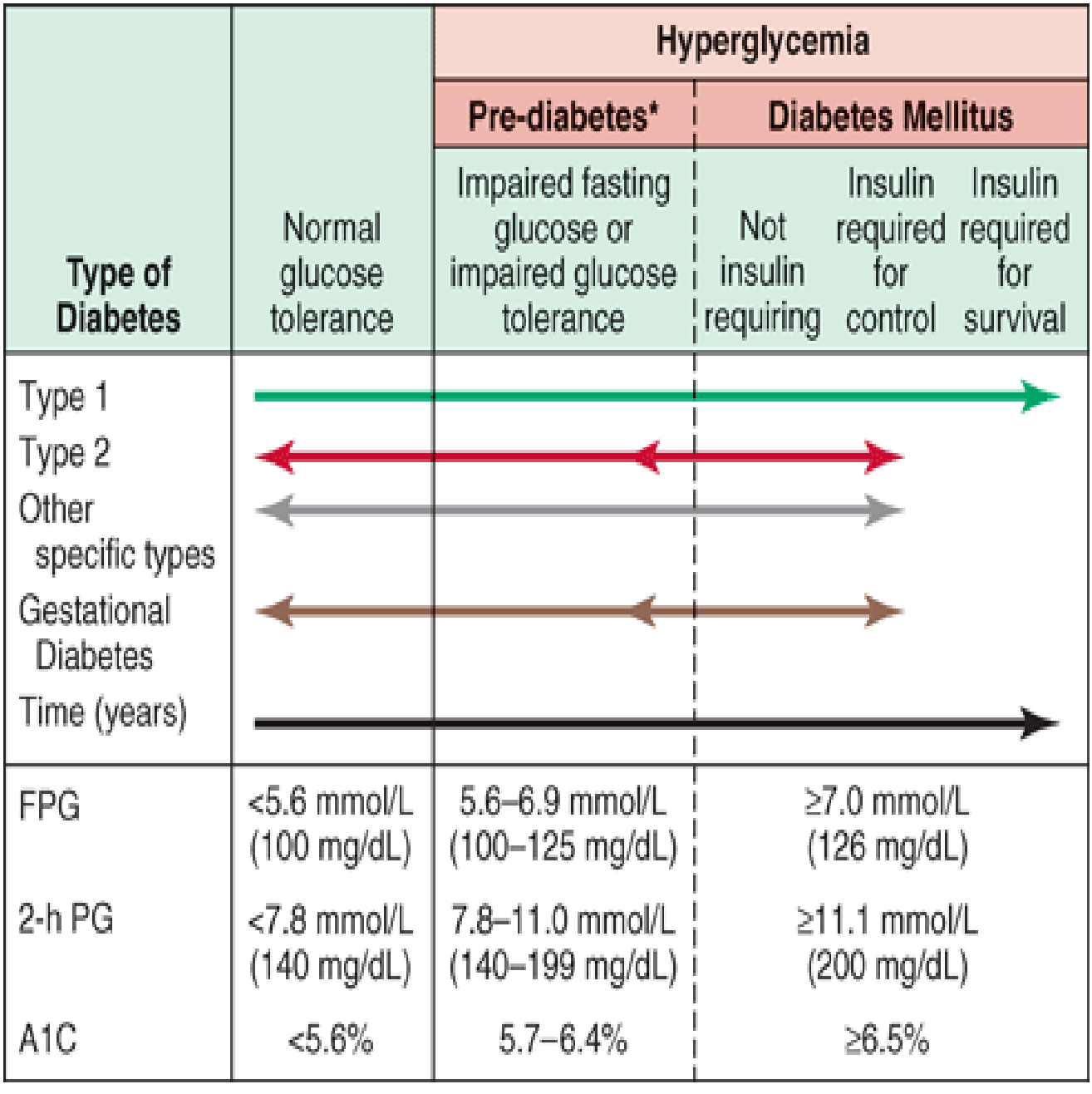
History of GDM or delivery of baby >4 kg (9 lb)

Hypertension (blood pressure ≥140/90 mmHg)

HDL cholesterol level <35 mg/dL (0.90 mmol/L) and/or a triglyceride level >250 mg/dL (2.82 mmol/L)

Polycystic ovary syndrome or acanthosis nigricans

History of cardiovascular disease



Source: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine, 18th Edition*: www.accessmedicine.com

TYPES:

The National Diabetes Data Group in 1979 created a scheme for classification and diagnosis of diabetes[50]. It divided diabetes into two major categories:

1. Type 1 IDDM
2. Type 2 NIDDM

The International Expert Committee on the Diagnosis and Classification of Diabetes sponsored by ADA revised the previous classification in 1995[51]

1. Type 1 DM
2. Type 2 DM
3. Other specific types
4. GDM

ETIOLOGICAL CLASSIFICATION OF DIABETES MELLITUS[175]

1. Type 1 diabetes (beta cell destruction, usually leading to absolute insulin deficiency) A. Immune-mediated B. Idiopathic

II. Type 2 diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly insulin secretory defect with insulin resistance)

III. Other specific types of diabetes

A. Genetic defects of beta cell development or function characterized by mutations in:

1. Hepatocyte nuclear transcription factor (HNF) 4α (MODY 1)

2. Glucokinase (MODY 2)

3. HNF-1α (MODY 3)

4. Insulin promoter factor-1 (IPF-1; MODY 4)

5. HNF-1β (MODY 5)

6. NeuroD1 (MODY 6)

7. Mitochondrial DNA

8. Subunits of ATP-sensitive potassium channel

9. Proinsulin or insulin

10. Other pancreatic islet regulators/proteins such as KLF11, PAX4, BLK, GATA4, GATA6, SLC2A2 (GLUT2), RFX6, GLIS3

B. Genetic defects in insulin action

1. Type A insulin resistance

2. Leprechaunism

3. Rabson-Mendenhall syndrome

4. Lipodystrophy syndromes

C. Diseases of the exocrine pancreas—pancreatitis, pancreatectomy, neoplasia, cystic fibrosis, hemochromatosis, fibrocalculous pancreatopathy, mutations in carboxyl ester lipase

D. Endocrinopathies—acromegaly, Cushing's syndrome, glucagonoma, pheochromocytoma, hyperthyroidism, somatostatinoma, aldosteronoma

E. Drug- or chemical-induced—glucocorticoids, vacor (a rodenticide), pentamidine, nicotinic acid, diazoxide, β-adrenergic agonists, thiazides, calcineurin and mTOR inhibitors, hydantoins, asparaginase, α-interferon, protease inhibitors, antipsychotics (atypicals and others), epinephrine

F. Infections—congenital rubella, cytomegalovirus, coxsackievirus

G. Uncommon forms of immune-mediated diabetes—"stiff-person" syndrome, anti-insulin receptor antibodies

H. Other genetic syndromes sometimes associated with diabetes— Wolfram's syndrome, Down's syndrome, Klinefelter's syndrome, Turner's syndrome, Friedreich's ataxia, Huntington's chorea, Laurence-MoonBiedl syndrome, myotonic dystrophy, porphyria, Prader-Willi syndrome

IV. Gestational diabetes mellitus (GDM)

TYPE 1

Constitutes only 5- 10% of all cases of diabetes. The disease begins before the age of 18 in approximately 75%cases[52]. Type 1 Diabetes is usually autoimmune in origin with the destruction of Islets of Langerhans of pancreas. Autoantibodies are directed against several particles of Islet cells encompassing antibodies to Insulin, Glutamic acid decarboxylase, tyrosine phosphatase IA-2 and IA- 2B [53]. Clinical features include polydipsia, polyphagia, polyuria, rapid weight-loss, hyperventilation, abrupt onset, insulin dependence and ketotic tendency[53].

IDIOPATHIC TYPE 1

Avidly inherited without β-cell autoimmunity. These patients require episodic replacement of Insulin.

TYPE 2

Hyperglycemia resulting from Insulin resistance due to secretory defect of Insulin will cause a relative but not absolute Insulin deficiency. Patients in this case are mostly obese with strong genetic predisposition and the risk increases with increasing age, obesity and physical inactivity. It is characterised by adult onset, with milder symptoms and rarely, when not treated properly, leads to ketoacidosis.

IMPAIRED GLUCOSE TOLERANCE [IGT]

It is observed in people whose fasting plasma glucose values are normal, with abnormal Glucose Tolerance Test. IGT is now termed as **Pre-Diabetes**. A

study shows that IGT is the intermediate step in the establishment of adult-onset type 2 diabetes mellitus.[54]

GESTATIONAL DIABETES MELLITUS (GDM)

Any level of glucose intolerance recognised for the first time during or after 24 weeks of pregnancy; must be considered as a risk factor for Type 2 DM. Etiology is related to metabolic changes of late pregnancy and its insulin requirements. Diabetes identified at the initial visit itself during pregnancy is diagnosed as "overt" diabetes. With rising prevalence of obesity the rates of diagnosing GDM or overt diabetes is soaring.

PATHOPHYSIOLOGY OF TYPE 2 DIABETES MELLITUS

AN OVERVIEW OF GLUCOSE HOMEOSTASIS

The balance among glucose production, peripheral glucose uptake and its utilization reflects Glucose Homeostasis. Although Insulin is the major controller of glucose metabolism, neural signals, metabolic inputs and other hormones (e.g. glucagon) also are involved. The organs that manage glucose and lipids interact by neural and humoral mechanisms with fat and muscle secreting adipokines, myokines, and metabolites that affect hepatic function. During fasting, low insulin increases glucose levels in blood by inducing hepatic gluconeogenesis and glycogenolysis and decreases uptake of glucose among insulin-sensitive organs (skeletal muscle and fat), thus mobilizing stored precursors as amino acids (proteolysis) and free fatty acids (lipolysis). When blood glucose or insulin levels are down, pancreatic alpha cells secrete

Glucagon which stimulates glycogenolysis and gluconeogenesis in the liver and renal medulla.

After a meal, the glucose load, rises Insulin and decreases Glucagon, reverses the aforesaid processes. Insulin, being an anabolic hormone, promotes the synthesis of carbohydrate, fat and protein. Major portion of post-meal glucose is used by skeletal muscle, which is an effect of insulin-stimulated glucose uptake. Other tissues, most notably the brain, use glucose in an insulin-independent fashion. Proteins secreted by skeletal myocytes (irisin), adipocytes (leptin, resistin, adiponectin, etc.), and bone also affect glucose homeostasis.

INSULIN BIOSYNTHESIS

Beta cells of the pancreatic islets produce Insulin. Initially, it is synthesized as a single-chain precursor polypeptide of 100 aminoacids, Pre-proinsulin. Subsequently proteolytic cleavage removes the amino-terminal signal peptide forming Proinsulin. Proinsulin, an 86 aminoacid structurally resembles insulin-like growth factors I and II, which weakly bind to the insulin receptor. Proinsulin is stored in golgi complex. Here it is cleaved into a 31-residue fragment, the C peptide and the A (21 amino acids) and B (30 amino acids) chains of insulin, are generated which are connected by disulphide bonds[64]. Finally, the mature insulin molecule and C peptide are stored together and secreted together from secretory granules in the pancreatic beta cells.

C peptide is excreted more slowly than insulin. So it is a useful marker of insulin secretion and allows discrimination between endogenous and

exogenous insulin in the evaluation of hypoglycemia. Pancreatic beta cells always secrete islet amyloid polypeptide (IAPP) or amylin, a 37-amino-acid peptide, along with insulin. The physiological role of IAPP is not completely defined, but it forms the major component of the amyloid fibrils deposited in the islets of patients with type 2 DM, and an analogue is sometimes used in the treatment of type 1 and type 2 DM. Human insulin is synthesised by recombinant DNA technology.

Insulin biosynthesis is controlled at the levels both transcription and translation. insulin content in β-cells is highly dynamic. In the presence of nutrients Insulin accumulates and decreases in response to nutrient deprivation. The quick response of β-cells to cellular signals is generally due to transcriptional regulation[63]

INSULIN SECRETION

Glucose is the most important regulator of insulin secretion, although amino acids, ketones, various nutrients, gastrointestinal peptides, and neurotransmitters also affect insulin secretion. Insulin synthesis is stimulated by a glucose level >3.9mmol/L (70mg/dL), primarily by promoting protein translation and processing. Stimulation of insulin secretion by glucose begins with its transport into the β-cells through a facilitative glucose transporter. Phosphorylation of glucose by glucokinase is the rate-limiting step that regulates glucose-mediated insulin secretion. Further steps of glycolysis generates ATP, which reduces the activity of an ATP-sensitive K+ channel.

This channel consists of two separate proteins: one is the binding site for certain oral hypoglycemics (e.g., sulfonylureas, meglitinides); the other is aK+ channel protein (Kir6.2). Inhibition of this K+ channel stimulates β-cell membrane to depolarize which opens up voltage-dependent calcium channels (causing calcium influx) and promotes insulin secretion. Secretory profiles of insulin reveal a pulsatile pattern of hormone release. Small secretory bursts occur about every 10 minutes, combined with greater amplitude oscillations of about 80–150 min. Incretins are secreted by neuroendocrine cells of the gastrointestinal tract after food ingestion and they improve glucose-influenced insulin secretion and suppress glucagon secretion. Glucagon-like peptide 1 (GLP-1), released from L cells in the small intestine is the most potent incretin. It induces insulin secretion only when the blood glucose level is above the fasting level. Incretin analogues are pharmacologic agents that increase the activity of endogenous GLP-1 enhanced insulin secretion.

INSULIN RESPONSE CURVE

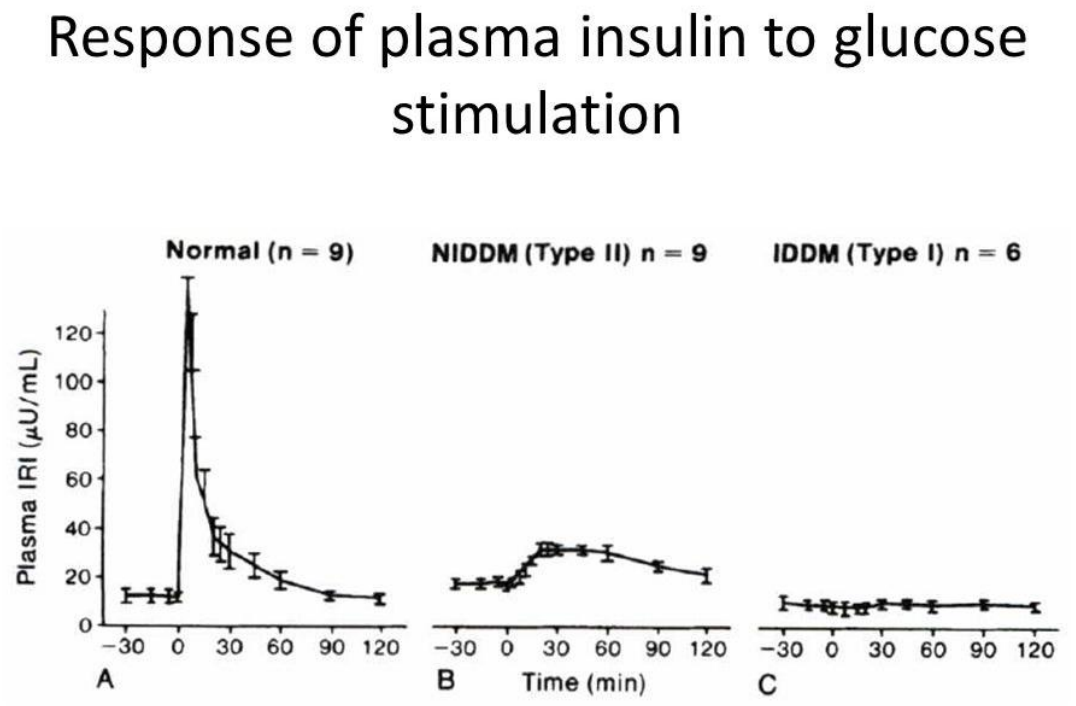
Insulin response occurs in two phases.

First phase insulin response: it occurs within the first ten minutes following a glucose injection. It is due to the release of stored insulin.

Second phase insulin response: begins just after the first-phase response has stopped. It is due to the continuing insulin synthesis and its release.

In most type 2 Diabetes patients the second phase response is conserved while the first phase response and the normal pulsatile insulin secretion are lost[(65)]. In type 1 Diabetes patients there is very low or nil insulin response.

Plasma insulin response to glucose administration. An intravenous pulse of 20g Insulin was administered at time 0. The values in the curve before time-0 indicate baseline values.



INSULIN ACTION

After insulin secretion into the portal venous system, ~50% is cleared and by the liver. The remaining insulin enters the systemic circulation and binds to specific receptors on target sites. The molecular basis of Insulin action is still not completely clear[66,67]. On binding with the insulin receptor intrinsic tyrosine kinase activity is stimulated, leading to receptor autophosphorylation and phosphorylation of intracellular signalling molecules, such as insulin receptor substrates (IRS 1,2,3 & 4; Shc & Gab-1). IRS and other adaptor proteins initiate a sequence of phosphorylation and dephosphorylation reaction, causing the widespread metabolic and mitogenic effects of insulin. One of the cascades is the activation of the phosphatidylinositol-3′-kinase (PI-3-kinase)

pathway leading to translocation of GLUT4 to the cell surface, resulting in glucose uptake by skeletal muscle and fat. Insulin activates other such signalling pathways inducing glycogen synthesis, protein synthesis, lipogenesis, and modification of various genes in insulin-responsive cells.

INSULIN LIKE GROWTH FACTORS

Two types of Insulin-like Growth Factors (IGF) are known. IGF 1 & 2 are polypeptides related to Insulin structurally.[68] The IGFs are found to be involved in cancer development as suggested by the accumulating recent evidences.[69] IGF1 mediates growth hormone action and is a major controller of cell growth and differentiation. The function of IGF 2 is not clearly defined. They exert their actions by binding with specific IGF 1 and 2 receptors. The circulating concentration of IGFs is 1000 times that of Insulin still they are kept inactive by binding with nearly six family of specific binding proteins[70]. The role of IGFs in normal metabolism of carbohydrates is not understood but administration of exogenous IGF produces hypoglycaemia. Inadequate IGF 1 causes dwarfism.

COUNTER REGULATORY HORMONES

GLUCAGON

It's a polypeptide hormone with 29 aminoacids. Glucagon induces glycogenolysis and gluconeogenesis in liver[145] and lipolysis in adipose tissue. Its secretion is primarily regulated by plasma glucose concentration. Low plasma glucose stimulates and high plasma glucose inhibits glucagon secretion.

GLUCAGON LIKE PEPTIDE (GLP)

GLP1 and GLP2 are produced by L-cells in the distal gut. GLP1 stimulates insulin gene transcription and enhances Glucose-induced insulin secretion in pancreatic -cells following a meal. Glucose-dependent Insulinotropic Polypeptides (GIP) and GLP-1 are incretin hormones and they are the reason for 70% of postprandial insulin secretion.[71] So GLP1 analogues are the drugs of recent interest in the treatment of type 2 DM.

EPINEPHRINE

A catecholamine, synthesised and released by the adrenal medulla induces glucose production and reduces glucose use. In type 1 diabetes, epinephrine plays a major role in counter-regulation of glucose when glucagon secretion is also affected. Physical and emotional stress raises production of epinephrine, thus releasing glucose for energy production.

GROWTH HORMONE

A polypeptide hormone secreted by the anterior pituitary gland. Inhibits insulin-mediated glucose uptake, induces gluconeogenesis and lipolysis.

CORTISOL

Secreted by the adrenal cortex, stimulates gluconeogenesis and breakdown of proteins and fats.

OTHER HORMONES AFFECTING GLUCOSE METABOLISM

THYROXINE

Thyroxine of thyroid gland stimulates glycogenolysis, enhances gastric emptying and improves intestinal glucose absorption. Thus, thyrotoxicosis patients may have glucose intolerance but the fasting plasma glucose is usually within the reference limits.

SOMATOSTATIN

Also known as the growth hormone- inhibiting hormone is found in the gastro-intestinal tract, δ-cells of pancreas and the hypothalamus. It affects carbohydrate metabolism indirectly by inhibiting the release of growth hormone. Somatostatin also modulates the reciprocal functions of glucagon and insulin.

PATHOPHYSIOLOGY

Two main pathological defects have been identified[57,58,59]

1. **Insulin resistance** – insulin's ability to act on peripheral tissues is reduced
2. **β-cell dysfunction**- pancreas cannot sufficiently produce insulin to match insulin resistance

It's now clearly established that type 2 DM is a heterogenous syndrome and there is no single cause for the incidence and progression of diabetes.

INSULIN RESISTANCE:

Defined as "a decreased biological response to normal concentrations of circulating Insulin"[60]. It is due to defective Insulin action. Insulin resistance can assume two spectra: **Euglycemic** (with high endogenous Insulin) and **Hyperglycemic** (despite large doses of exogenous Insulin).[61]

Insulin Resistance Syndrome (Metabolic syndrome/ syndrome X)

Its established if a person meets three or more of the following criteria[61]

- Abdominal obesity (waist circumference >35 inches in females or >40 inches in males)
- TGL >150mg/dL
- HDL <50mg/dL (females) or <40mg/dL (males)
- Blood pressure ≥130/85 mmHg
- Fasting plasma glucose ≥110mg/dL

LOSS OF β-CELL FUNCTION:

Hyperglycemia over a period of time renders the β-cells unresponsive to its increase. This is termed **glucotoxicity**[120,121,72]. The degree of unresponsiveness correlates with glucose concentration and duration of hyperglycemia. But reverting to euglycemia rapidly restores the defect. Increased serum fatty acid has also been found to be involved in the β-cell failure[72]. Recent evidences suggest that insulin resistance could lead to alterations in the β-cell production of insulin, as in type 2 diabetes [73].

ENVIRONMENT

Diet and exercise are the most important environmental factors implicated in the pathogenesis of type 2 Diabetes. Besides the fact that 60- 80% of diabetic individuals are obese, Diabetes develops only in less than 15% of obese people. But almost all obese individuals are hyperinsulinemic and have insulin resistance. In some randomized studies life-style changes (enhanced physical activity) in subjects with IGT decreased the incidence of type 2 diabetes.[74, 75]

With every 500kcal raise in energy expenditure in a day, a reduction of 6% in age adjustable risk for type 2 diabetes has been noticed[146]. This is because physical activity enhances the sensitivity of skeletal muscles and adipose tissue to insulin.

GENETIC FACTORS

Studies done with identical twins, off-springs and twins show clearly that Diabetes Mellitus develops from a complicated interplay of genetic and environmental factors.[55,56]

Type 1: the incidence in one twin increases the chance in the other by 50%

Type 2: the incidence in one twin increases the chance in the other by nearly 100%

The incidence of type 2 diabetes in an obese individual with a diabetic parent is 10 times more in an equally obese individual without a diabetic family history. But the mode of inheritance is still unclear and so type 2 diabetes has

been named as "**geneticists' nightmare**"[76]. It is caused by multiple genetic factors with environmental influence.

Several mutations involving the insulin receptor gene (INSR) have been identified[77]. But these mutations are too rare and these patients experience extreme levels of insulin resistance. Numerous factors contribute to the difficulty in searching the diabetogenes in type 2 diabetes[78]. Though there has been so much efforts undertaken still the diabetogenes identified to date account for only around 5% of the patients identified with type 2 diabetes[147]. Thus the genetic defects causing the common forms of type 2 diabetes are yet to be identified.

OMINOUS OCTET

The pathogenesis of type 2 diabetes has broadened from the triumvirate of β-cell–, muscle-, and liver-related defects[153] to the "ominous octet" described in the 2008 Banting Lecture[154]. The most recent and important player in the pathogenesis of Type 2 Diabetes Mellitus is the brain. The brain and its seven accompaniments form the "Ominous Octet".

The components and their mechanisms in the pathogenesis of type 2 DM are

Muscle- Decreased glucose uptake

Adipose tissue- Increased lipolysis

Pancreatic β-cells- Reduced Insulin production

Liver- Increased glucose synthesis

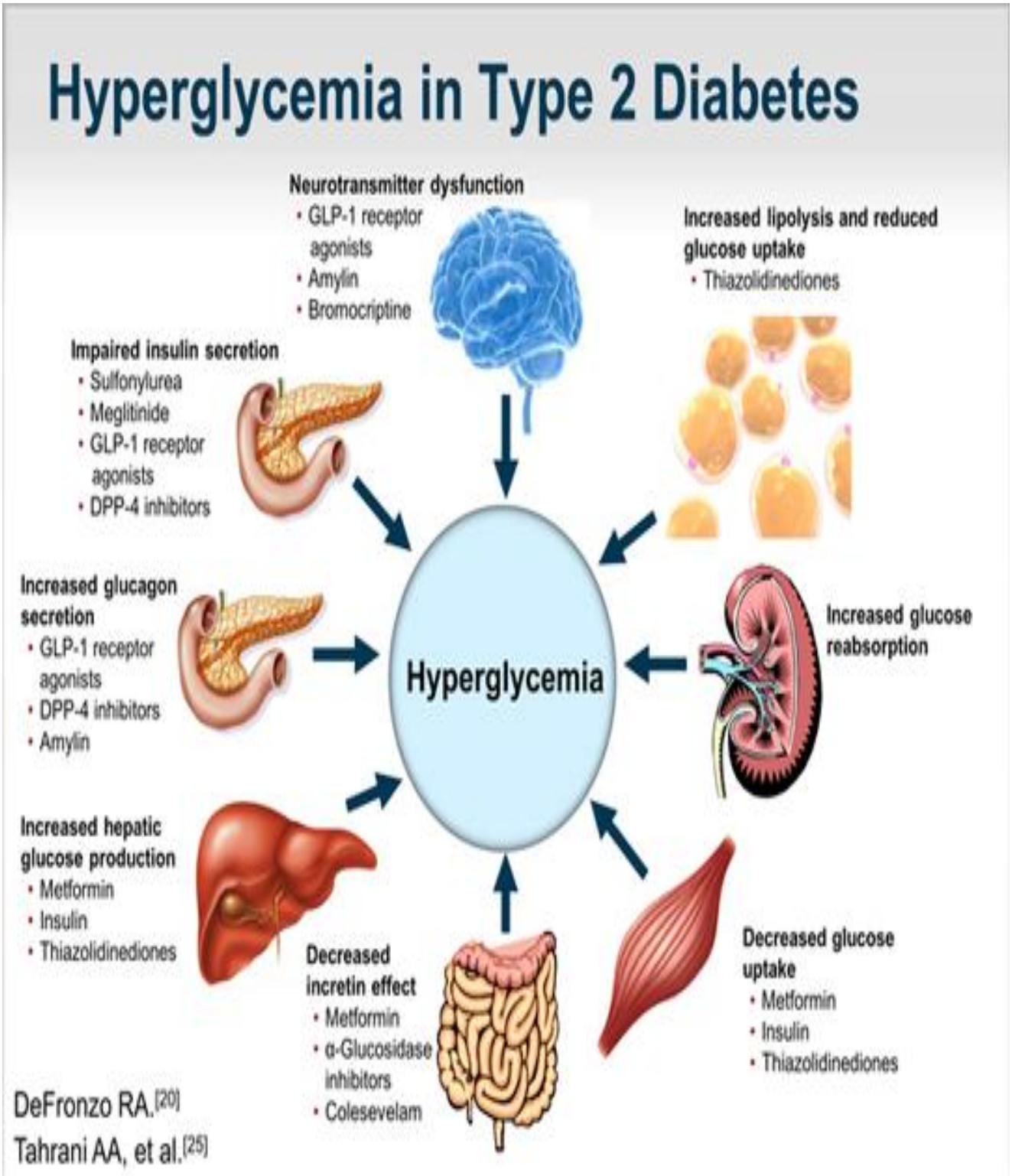
Brain- Neurotransmitter dysfunction

Pancreatic α-cells- Increased glucagon production

Kidney- Increased glucose reabsorption

Intestine- reduced Incretin effect

It was demonstrated by Porte and colleagues that in rodents Insulin was a powerful appetite suppressant[149,150]. It is well established that obesity is frankly associated with type 2 DM[151,152].Obese individuals, whether diabetic or nondiabetic, are insulin resistant and hyperinsulinemic. In spite of hyperinsulinemia their food intake is increased. This indicates that the insulin resistance extends from peripheral tissues to brain as well.


Hyperglycemia in Type 2 Diabetes
Neurotransmitter dysfunction
• GLP-1 receptor agonists
• Amylin
• Bromocriptine
Increased lipolysis and reduced glucose uptake
• Thiazolidinediones
Impaired insulin secretion
• Sulfonylurea
• Meglitinide
• GLP-1 receptor agonists
• DPP-4 inhibitors
Increased glucagon secretion
• GLP-1 receptor agonists
• DPP-4 inhibitors
• Amylin
Hyperglycemia
Increased glucose reabsorption
Increased hepatic glucose production
• Metformin
• Insulin
• Thiazolidinediones
Decreased incretin effect
• Metformin
• α-Glucosidase inhibitors
• Colesevelam
Decreased glucose uptake
• Metformin
• Insulin
• Thiazolidinediones
DeFronzo RA.[20]
Tahrani AA, et al.[25]

COMPLICATIONS OF TYPE 2 DIABETES

Complications of type 2 Diabetes can be

1. **Acute**
2. **Chronic**

The two main factors responsible for complications are elevated blood glucose levels for a long duration and repeated acute fluctuations in their levels[91]. They both worsen the morbidity through two principal mechanisms: (1) increasing oxidative stress and (2) altering the innate immune system [92,93].

Acute complications are due to sudden fluctuations of glucose levels in the blood, butin chronic complications vascular etiology plays a major role.

ACUTE COMPLICATIONS

HYPOGLYCEMIA

The most common complication and the most limiting factor in the treatment of type 2 DM is hypoglycemia. It is often due to antidiabetic drugs and insulin administration. The sympatho-adrenal response in diabetic patients are impaired and it can lead to “hypoglycemic unawareness” which will further complicate the situation. Frequent occurrence of hypoglycaemia will affect the efficient control of blood glucose during the treatment of the disease.

HYPEROSMOLAR NON-KETOTIC STATE

When blood glucose levels reach a hike (typically above 300mg/dL), due to severe insulin resistance, the blood sugar draws all the water from the

cells osmotically and cause dehydration. This causes osmotic diuresis and glucosuria in kidneys. There is hyperosmolality in the blood with hypernatremia. All these changes cause weakness, lethargy progressing to obtundation and coma as the blood sugar reaches abnormally high values of 800- 2400mg/dL. Absence of fruity breath and abdominal pain distinguishes it from ketosis and also there is no acidemia.

DIABETIC KETOACIDOSIS (DKA)

It is more common in type 1 diabetes and many times it may be the presenting illness itself. In type 2 diabetes DKA occurs only when there is severe insulin secretory defect precipitated by an acute stress like severe infection, burns, surgery. Insulin deficiency impairs peripheral glucose uptake by the muscles and adipose tissues. This leads to mobilization of the stored fat and proteins, in the form of fatty acid and aminoacids to the liver for producing glucose and ketones (β- hydroxybutyrate and acetoacetate primarily).

In order to compensate for the perceived hypoglycaemia, counter-regulatory hormones like glucagon, glucocorticoids, epinephrine, thyroxine increase in blood and further aggravate the hyperglycemia and ketosis. Hyperglycemia causes osmotic diuresis leading to hyperosmolality (>320 mosm/kg) and compromised renal function leads to acidosis. Patients thus present with fruity breath (due to the exhaled ketones), weakness, fatigue, abdominal tenderness with symptoms of underlying infections like urinary tract infections or respiratory infections. When the ketoacidosis and hyperglycemia

reaches too high levels there is altered mental status. They are treated with intravenous insulin and slow potassium infusions.

CHRONIC COMPLICATIONS

Chronic complications can be microvascular or macrovascular and are due to hyperglycemia over a long duration of time.

MICROVASCULAR

The microvascular complications of type 2 diabetes include diabetic neuropathy, nephropathy, retinopathy. The prevalence of overt nephropathy was 2.2%, diabetic retinopathy was 17.5%, neuropathy was 25.7% and microalbuminuria was 26.5% among diabetics of South India[(79)].

PATHOPHYSIOLOGY OF MICROVASCULAR EVENTS

- Cell injury is the main cause of microvascular complications. Cell injury can be from advanced glycation end-products formed from Millard reaction[86] they get deposited on arteries, brain, heart and kidney normally during ageing but hyperglycemia hastens and aggravates the process.
- Oxidative stress mediated cell injury due to hyperglycemia also complicates diabetes. The reactive oxygen species formed during the oxidative stress damages blood vessels and it is linked to microaneurysm formation[87].
- Hyperglycemia shunts the glucose utilisation through polyol pathway. The sorbitol collected intracellularly by the action of aldose reductase

produces an osmotic stress inside the cell. This forms the basis of retinopathy[88].

- Growth factors like VEGF, Somatotropic hormone, TGF-β are also involved in microvascular complications[89].

MACROVASCULAR

Macrovascular complications include Cerebrovascular disease (CVD), Coronary-Artery Disease (CAD), Peripheral Neural Disease (PND).

Approximately one half of patients with type 2 diabetes die prematurely of a cardiovascular cause and approximately 10% die of renal failure[80].

PATHOPHYSIOLOGY OF MACROVASCULAR EVENTS

The leading cause for macrovascular complications is atherosclerosis which causes obliteration of vessel walls.

- The vessel-wall injury caused by hyperglycemia, secondary hyperlipoproteinemia and increased free radicals triggers a cascade of events leading to atherosclerosis. It causes inflammation on the endothelium and lipids from LDL get deposited along with platelets and fibrin clot forming atherosclerotic plaque[87].
- Inefficient Nitric oxide production and dysregulated calcium metabolism enhances platelet aggregation[90].
- High plasminogen activator inhibitor concentration in type 2 Diabetes mellitus reduced fibrinolysis[87]

OPHTHALMOLOGIC COMPLICATIONS

The leading cause of blindness between the ages of 20 and 74 is DM. It is found that individuals with T2DM are 25 times more likely to become legally blind than individuals without it. Progressive diabetic retinopathy and clinically significant macular edema cause severe visual impairment.

Diabetic retinopathy is classified into two stages:

- Non-proliferative
- Proliferative

Non-proliferative diabetic retinopathy commonly appears late in the first decade or early in the second decade of the disease. It shows retinal vascular micro-aneurysms, cotton-wool spots and blot hemorrhages.

Proliferative diabetic retinopathy is marked by the appearance of neovascularization in response to retinal hypoxemia. These vessels are formed near the optic nerve and/or macula. They are very fragile and breakdown easily leading to vitreous haemorrhage, fibrosis and retinal detachment finally.

RENAL COMPLICATION OF DIABETES MELLITUS

Diabetic nephropathy is the major cause for end-stage renal disease(ESRD) and chronic kidney disease(CKD). As in other microvascular complications, the pathogenesis of diabetic nephropathy is also due to chronic hyperglycemia. Chronic hyperglycemia elicits its effect through soluble factors (growth factors, angiotensin II, endothelin, advanced glycation end products

[AGEs]), alterations in the renal microcirculation (glomerular hyperfiltration or hyperperfusion, increased glomerular capillary pressure), and structural changes in the glomerulus (increased extracellular matrix, basement membrane thickening, mesangial expansion, fibrosis). Smoking hastens the above processes.

The terms used to refer to increased urinary protein are

- "microalbuminuria"- 30–299 mg/d in a 24-h collection or 30–299 μg/mg creatinine in a spot collection
- macroalbuminuria as defined as >300 mg/24 h

The American Diabetes Association now recommends that the above terms be replaced by the phrases **"persistent albuminuria- (30–299 mg/24 h)"**[148]

The nephropathy that develops in type 2 DM exhibits the following characteristics

(1) Microalbuminuria or macroalbuminuria may be already present at the time of diagnosing type 2 DM, indicating its long asymptomatic period;
(2) Microalbuminuria or macroalbuminuria is more commonly accompanied by hypertension in type 2 DM;
(3) Microalbuminuria may be less predictive of likelihood of progression to macroalbuminuria in type 2 DM, mostly due to increased cardiovascular mortality in this population.

It should also be noted that albuminuria in type 2 DM may be secondary to causes unrelated to DM, like hypertension, congestive heart failure (CHF), infection or prostate disease.

DIABETIC NEUROPATHY

As with other microvascular complications of T2DM, neuropathy also is correlated well with the duration of diabetes and glycemic control over the period. It may be polyneuropathy, mononeuropathy and/or autonomic neuropathy. Distal symmetrical polyneuropathy is the most common manifestation. Mononeuropathy is very rare and its pathogenesis is not clearly understood yet.

Gastroparesis and abnormalities in bladder-emptying are the common manifestations of autonomic neuropathy. Autonomic neuropathy causes defective or nil release of counter-regulatory hormones during a hypoglycemic episode. This leads to hypoglycemic unawareness, a fatal complication. Upper extremities exhibit hyperhidrosis while lower extremities exhibit hyperhidrosis due to dysfunction of sympathetic nervous system.

LIPID ABNORMALITIES IN DIABETES MELLITUS

In type 2 diabetes Plasma lipoprotein profiles are often abnormal reflecting an elevation in the level of the apoprotein B (ApoB)-containing components; namely very low density lipoprotein (VLDL) and low density lipoprotein (LDL)[81]. High levels of circulating advanced glycation end products (AGEs) also occur in diabetes. AGE-modified LDLs[81] circulate in

the blood of diabetics and all these damage β-cells. The defect so produced is reversible till it reaches a stage where pancreatic β-cells become exhausted due to lipotoxicity and glucotoxicity[82,83]. The mechanism of beta-cell damage by glucotoxicity and lipotoxicity is through generation of free radicals[84]. Palmitate exposure generates reactive oxygen species in the islets and treatment with metformin (which has antioxidant properties) protects it from the deleterious effect of the fatty acid[85].

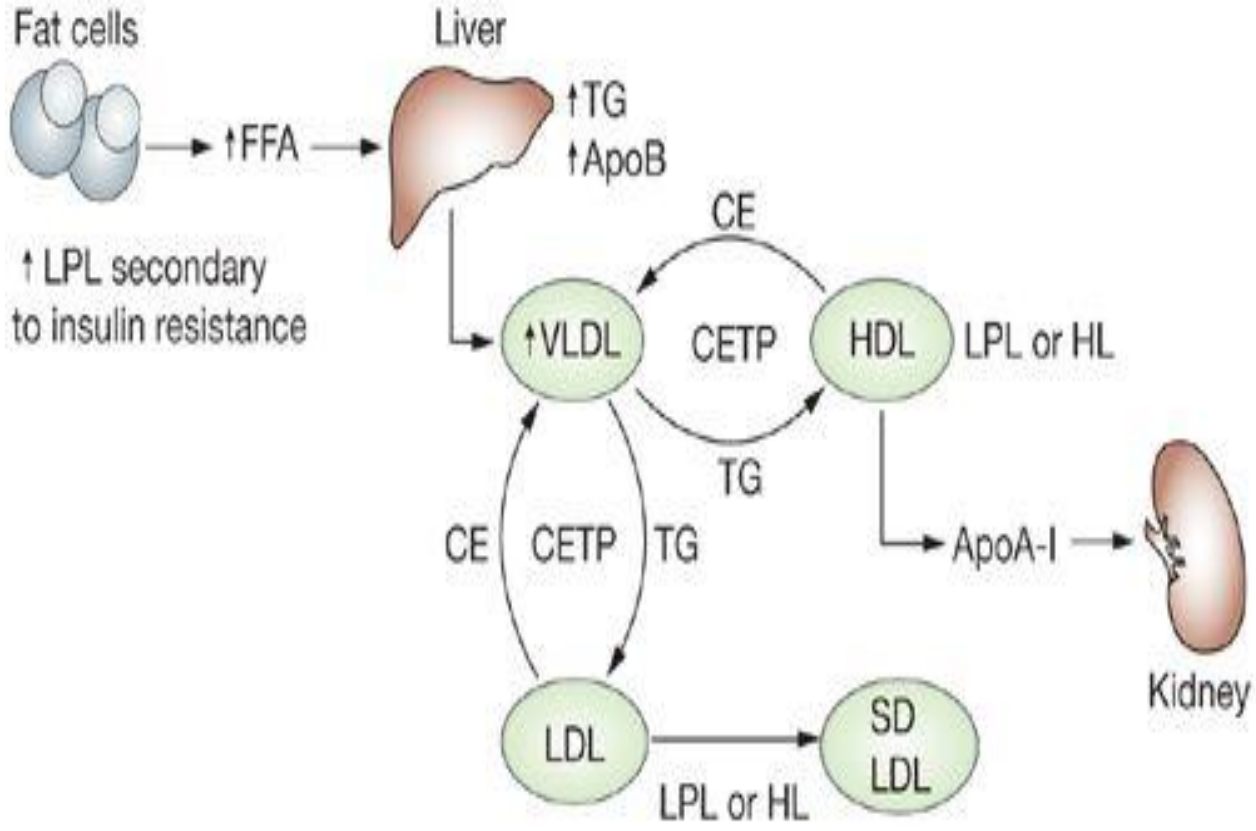
Insulin resistance of type 2 Diabetes in the liver shows failure of hyperinsulinemia to suppress gluconeogenesis. This causes fasting hyperglycemia and depleted glycogen storage by the hepatocytes in the postprandial state. Insulin resistance in adipose tissue results in lipolysis and an increase in free fatty acid mobilized from adipocytes, leading to high levels of lipid (very-low-density lipoprotein [VLDL] and triglyceride) synthesis in liver. This leads to non-alcoholic fatty liver disease and dyslipidemia found in type 2 DM (elevated triglycerides, reduced high-density lipoprotein [HDL], and increased small dense low-density lipoprotein [LDL] particles)[96]**.**

ROLE OF DYSLIPIDEMIA IN COMPLICATIONS OF TYPE2 DIABETES

The typical dyslipidemia of type 2 DM is elevated triglycerides, reduced high-density lipoprotein [HDL], and increased small dense low-density lipoprotein [LDL] particles[96]. Complications of type 2DM are associated with hypertension and dyslipidemia and both are known to influence small-artery structure and function[136,137]

Insulin resistance plays a key role in the diabetic dyslipidemia by allowing increased lipolysis in the adipose tissue and decreased free fatty acid in the skeletal muscles. This leads to an overall increase in the free fatty acid level in circulation and are fluxed to liver[162,163]. Here there is increased secretion of VLDL and defective clearance of VLDL and intestinal chylomicrons. The prolonged retention of VLDL and chylomicrons leads to partially lipolyzed remnants in circulation. They are the Intermediate Density Lipoproteins (IDLs) and are cholesterol-rich and are specifically atherogenic[164.165]. The above explained increased secretion and defective clearance of VLDL also leads to the production of small dense LDL[166]. Small dense LDL shows high atherogenic potential due to a number of factors including reduced affinity for LDL-receptor[167,168] greater binding to arterial wall proteoglycans[169] and vulnerability to oxidative changes[170,171].

MECHANISM RELATING DYSLIPIDEMIA AND T2DM



CE: Cholesterol Ester; **CETP:** Cholesterol Ester Transfer Protein**; SD LDL:** Small-Density Low Density Lipoprotein**; FFA-** Free Fatty Acid

Reduction in the HDL level associated with type 2 diabetes is mainly because of the increased relocation of cholesterol from HDL to LDL and VLDL along with transfer of triglycerides to HDL. Liver rapidly clears these HDL particles rich in triglycerides by hepatic lipase[172]. Evidences claim that elevation of free fatty acids could occur earlier than the onset of hyperglycemia in diabetic individuals[173].

For every mmol/L increase in plasma triglyceride level there is a 32% increase in coronary artery disease in men and 76% increase in women, as found by the meta-analysis of 17 population-based prospective studies[174].

It has been recognized that hypercholesterolemia obliterates the vessel-diameter by reducing the bioavailability of nitric oxide through the following mechanisms:

- Decreased availability of L-arginine
- Nitric oxide activation through downregulation of $G_{i\alpha}$ subunit of the G-protein coupled receptor.
- Reduced expression of nitric oxide synthase
- Inhibition of the effect of nitric oxide by superoxide anions or oxidized lipoproteins[138-141].

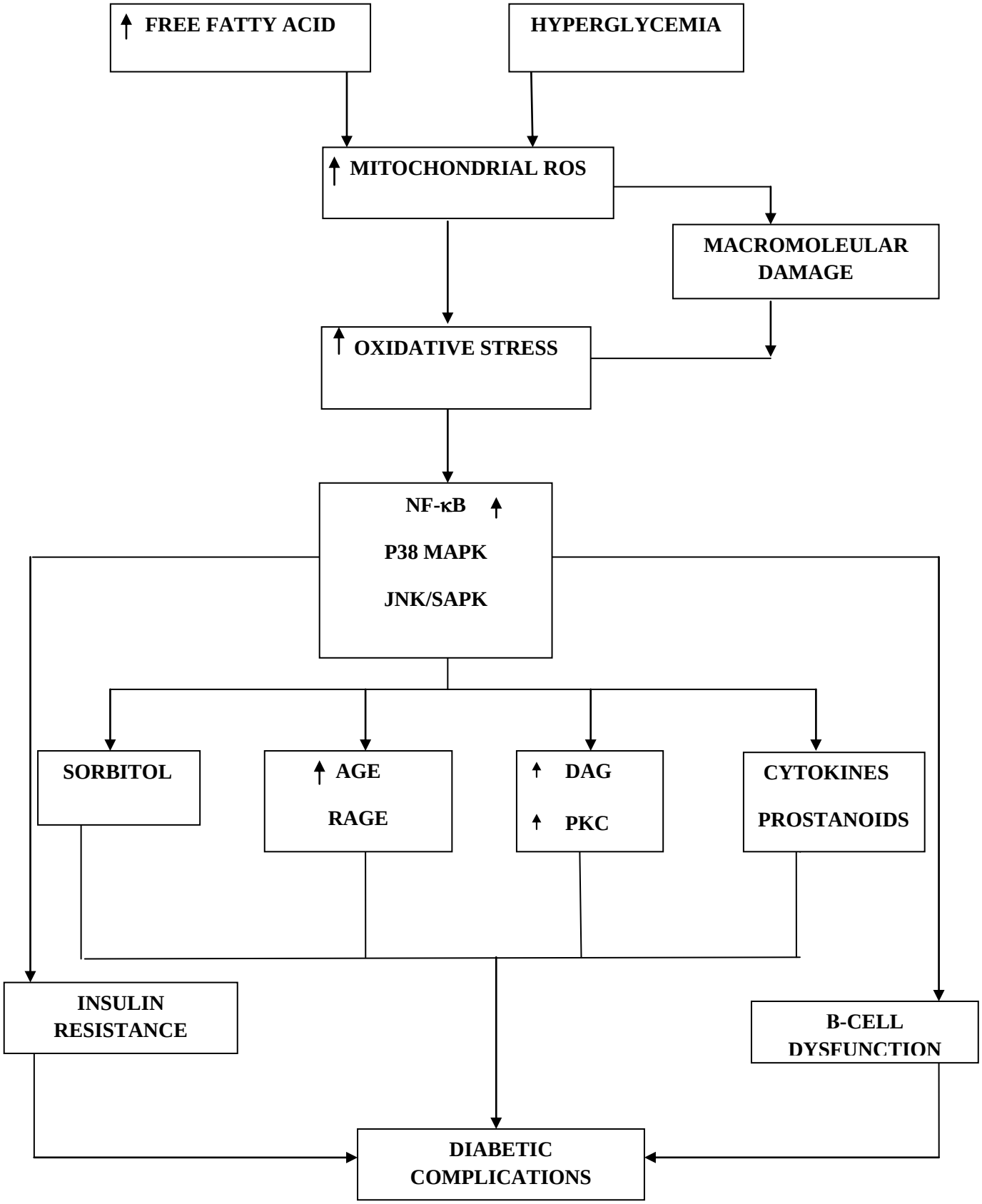
It is observed that there is small reduction of lumen diameter and an increase in medial thickness without a change in cross-sectional area[142]. Increased wall stress is seen in the diabetic resistance vasculature as a result of an impaired myogenic response which may lead to vessel-wall hypertrophy.

This explanation indicates that changes in small-artery structure succeeds defect in the myogenic response of the vessels.

FREE RADICAL GENERATION IN TYPE 2 DIABETES

Insulin resistance in skeletal muscles causes piling up of lipids within skeletal myocytes with defective mitochondrial oxidative phosphorylation and decreased insulin-stimulated mitochondrial ATP production. Defective fatty acid oxidation and lipid accumulation inside the skeletal myocytes generates reactive oxygen species like lipid peroxides[96]. Reactive oxygen species and free radicals generated during aerobic metabolism of glucose is also implicated in the cellular damage of most cells[94,95]. It has been found that the key process in the induction of all the events involved in the development of diabetic complications is a single hyperglycemia-induced pathway of overproduction of superoxides by electron-transport chain[98]. There is high nitric oxide production along with Superoxide overproduction due to an endothelial-NOS and inducible-NOS uncoupled state (a state that favors the production of strong pro-oxidant peroxynitrite). The pro-oxidant damages DNA which is an obligatory activator of the nuclear enzyme poly(ADP-ribose) polymerase. Active Poly(ADP-ribose) polymerase consumes the intracellular NAD(+), its substrate. This slows down the rate of glycolysis, electron transport, and ATP formation, producing ADP-ribosylation of the GAPDH [99]. All these lead to acute endothelial dysfunction of diabetic blood vessels that contributes to the development of diabetic complications[97,100].

Proposed mechanism by which hyperglycemia and elevated free fatty acids could cause complications in a type 2 Diabetes patient



OXIDATIVE STRESS

Though it is well-established that obesity and physical inactivity are the major risk factors for type 2 diabetes (T2DM), recent evidence indicate that oxidative stress may contribute to the pathogenesis of T2DM by affecting normal insulin secretion or by increasing insulin resistance[103]. As described above hyperglycemia generates reactive oxygen species and it also afflicts the endogenous antioxidant capacity in diabetics[101]. These untamed reactive oxygen species (ROS) denature some of the essential functional proteins of cell and cause cellular dysfunction[102]. Hyperglycemia leads to increased production of reactive oxygen species or superoxide in the mitochondria; these compounds may increase the production of Advanced Glycated End-products; the transcription of genes involved in VEGF, TGF-β, Plasminogen Activator Inhibitor-1; enhance glucose utilization via sorbitol pathway; promotes production of diacyl glycerol which alters transcription of genes for contractile proteins, fibronectin, extracellular matrix proteins and type IV collagen in endothelial cells and neurons[104].

The main burden of type 2 Diabetes is it its vascular complications as shown be the 4-time increased incidence of coronary artery disease; 10-fold rise in peripheral vascular disease and 3 to 4-time higher mortality rate seen in about 75% diabetics dying from vascular diseases[105]. Hyperlipidemia, hyperinsulinemia and hyperglycemia associated with type 2 Diabetes act as “triggers” leading to endothelial dysfunction via “mediator-molecules”.

Increasing evidences indicate that the “oxidative stress” due to the above said metabolic changes play an important role in endothelial dysfunction[106].

ROLE OF ANTIOXIDANTS IN TYPE 2 DIABETES

An imbalance between oxidative free radical generation and the neutralizing antioxidants results in Oxidative Stress[107,108].

Antioxidants function by four stages:

- Prevention of formation of free radicals
- inhibition of the effects of free radicals once they are formed
- repairing damages caused inflicted by the free radicals
- they enhance the removal of damaged molecule[109]

ENDOGENOUS ANTIOXIDANTS

There are two types:

- Enzymatic
- Non-enzymatic

ENZYMATIC ANTIOXIDANTS: Superoxide dismutase (SOD), glutathione peroxidase (GPO) and catalase are the most important enzymatic antioxidants[110]Reports claim that there is sever disturbance of peroxidase activity, free-radical production and antioxidant defences in diabetes and pregnancy[111].

NON-ENZYMATIC ANTIOXIDANTS: In human body they are contributed by glutathione, uric acid (at physiological levels), bilirubin. Exogenous non-

enzymatic antioxidants include ascorbate, α-tocopherol, γ-tocopherol, β-carotene, α-carotene, Lutein, Lycopene, Ubiquinol-10[112]

Antioxidant therapy combined with blood pressure control, optimal glucose control and management of dyslipidemia is beneficial in the treatment of macrovascular/microvascular complications[113]. The antioxidant pharmacotherapy comprises the use of antioxidant enzyme and substrates, synthetic antioxidants, biogenic elements and drugs with antioxidant activity. Exercise training up-regulates antioxidant defence mechanisms in various tissues most probably due to increased levels of oxidative stress that occurs during exercise[114].

Vitamins C, E, A, and carotenoids are some of the well-established anti-oxidants found in diet. Evidences accumulated over the past decade imply that plant polyphenols form an important group of defence antioxidants. These compounds are found virtually in all plant foods at very high levels and they encompass phenols, flavonoids and phenolic acids[115].

ROLE OF LIFESTYLE MODIFICATIONS

An interaction between a genetic predisposition with behavioural and environmental risk factors causes type 2 diabetes[116]. Increasing evidences suggest that such modifiable risk factors include obesity and physical inactivity[117-124].The results from studies conducted in Sweden[125] and China[126] prompt that changes in lifestyle not only improved glucose tolerance

significantly but also reduced the effect of several other cardiovascular risk factors[127].

Lifestyle modification (LSM) involving diet and enhanced physical activity helps to delay or prevent the progression of IGT to diabetes. This is proved by many prospective randomised controlled studies including the Diabetes Prevention Program (DPP) in the USA[128] the Finnish Diabetes Prevention Study[129] (DPS), the Da Qing IGT and Diabetes Study in China[130]and the Malmo study in Sweden[131].In the DPP, LSM produced similar effects in Asian and other ethnic groups[128].

ROLE OF LIPID LOWERING THERAPY

There are growing medical evidences claiming that there is 22- 24% reduction in major cardiovascular events in type 2 diabetes patients. The typical lipid profile a type 2 diabetes patient shows high triglyceride level (TGL), low high-density lipoprotein (HDL) and average low-density lipoprotein levels[132,133]. Lipid-lowering agents are important in modifying cardiovascular risk effectively and its more cost-effective[134,135].The commonest complication of type 2 diabetes is cardiovascular disease contributing to 80% of diabetic patients developing some type of major vascular event[174]. Thus, therapy in type 2 diabetes should be channelled towards preventing cardiovascular disease through risk factor modification. This includes smoking cessation; effective treatment of hypertension[134];aspirin therapy; aggressive lipid-lowering therapy, particularly with 3-hydroxy-3-methylglutaryl coenzyme A reductase

inhibitors (statins). Analyses by the Centers for Disease Control and Prevention using a simulation model based on Framingham data and the United Kingdom Prospective Diabetes Study[143] and another by Grover and colleagues using data from a Canadian cohort[144] found that lipid-lowering therapy was reasonably cost-effective when compared with other commonly adopted medical interventions.

ATHEROGENIC INDEX OF PLASMA

Efforts have been continuously made using clinical studies to develop a standard marker of atherogenic dyslipidemia(155). Atherogenic Index of Plasma (AIP) has been shown to be a strong marker in predicting risk for coronary artery disease and atherosclerosis(156,157,158)because of its powerful association with lipoprotein particle size(159). AIP is calculated as logarithm of ratio of molar concentration of TGL to the molar concentration of HDL(161,156,157,158).

$$\text{Atherogenic Index of Plasma} = log \frac{\text{(molar concentration of TGL)}}{\text{(Molar concentration of HDL)}}$$

The predictable risk values are(159,160)

Low risk <0.11

Intermediate risk 0.11- 0.21

High risk >0.21

MATERIALS AND METHODS

AIM: To compare metabolic risk factors and Total Antioxidant Capacity in Diabetics and healthy individuals

OBJECTIVES:

1. To assess the metabolic risk factors by measuring parameters like fasting and post-prandial blood sugar, HbA_{1C}, Lipid profile, AIP

2. To assess the Total Antioxidant Capacity

STUDY CENTRE This study was conducted in the department of Biochemistry clinical laboratory at Government Kilpauk Medical College, Kilpauk, Chennai-10 during the period of December-2017 to march-2018

STUDY DESIGN Case-Control study

GEOGRAPHIC DISTRIBUTION includes only the urban population presenting at the Government Kilpauk Medical College

SAMPLE SIZE 50 diabetics (type-2 DM) and 50 controls

CASES

INCLUSION CRITERIA

1. patients diagnosed with type 2 DM irrespective of the duration of treatment
2. age group of 40- 60 years
3. both genders

EXCLUSION CRITERIA

1. Type 1 Diabetes Mellitus
2. Patients with micro and macrovascular complications
3. Pregnancy
4. Lactation
5. Individuals with history of alcoholism/ smoking
6. Patients with malignancy
7. Patients with Arthritis, Cardiac and Renal diseases
8. Patients with history of smoking and alcohol

CONTROLS: Gender and age-matched healthy controls

ETHICAL CLEARANCE We got the approval to conduct the study at Government Kilpauk Medical college from the Institutional Ethics Committee. Patients were explained about the study. Informed and explained written consent was obtained. Confidentiality was maintained and the participation of patients was purely optional.

PROCEDURE

Case Record Form(CRF) was prepared in such a way it included all the criteria to fulfil the objectives of the study.

Each person was evaluated as follows,

1. Detailed history as per CRF
2. Duration of type-2 Diabetes

3. Occupation
4. Diet
5. Measured for height and weight and BMI was calculated

SAMPLE COLLECTION

3mL venous blood sample was collected in a red tube and 2mL venous sample collected from the same puncture site with a heparinised tube.

Samples were collected between 7-9 am after a 10-hour fasting and immediately centrifuged. The plasma of the samples were separated and stored at -20 and the heparinized plasma were stored at -60

METHODOLOGY

ESTIMATED PARAMETERS

1. Fasting blood glucose -by Glucose oxidase-peroxidase method
2. HbA_{1c} -particle enhanced immunoturbidimetry
3. LIPID PROFILE
    - Serum Total Cholesterol- Cholesterol Oxidase- Peroxidase
    - Serum Triglycerides - Glycerol 3-phosphate Oxidase
    - Serum HDL-c - Direct Enzymatic method

CALCULATED PARAMETERS

Body Mass Index (BMI) is calculated by

$$\text{BMI } (Kg/m^2) = \frac{\text{Weight (kg)}}{\text{Square of Height}(m^2)}$$

ESTIMATION OF FASTING BLOOD SUGAR

METHODOLOGY

Glucose Oxidase Peroxidase method (end point)

PRINCIPLE

Glucose on oxidation gives Gluconic acid by the enzyme Glucose Oxidase releasing Hydrogen Peroxide, which on further reaction by Peroxidase releases nascent oxygen and water by the enzyme peroxidase. 4-Aminoantipyrine takes up the oxygen, simultaneously with phenol, forms pink colored Chromogen which is measured at 505nm.

$$\text{GLUCOSE} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{GLUCONIC ACID} + H_2O_2$$

REAGENTS:

ASSAY PROCEDURE: (FULLY AUTOMATED ANALYSER)

Procedure:

The glucose reagent is brought to room temperature (27℃). Pipette the reagent into test tubes and labelled as blank (B), test (T) and standard (S) as follows

S.No	REAGENT	BLANK	STANDARD	TEST
1	Glucose reagent	1.0mL	1.0mL	1.0mL
2	Glucose Standard	--	1.0µL	--
3	Specimen	--	--	10µL

Incubation period- 10 minutes/ Temperature- 37°C

Mixed well and absorbance of Test (T) and standard (S) was read at 505nm

CALCULATION:

Glucose concentration (mg/dL)= Δ Abs- Test/ Δ Abs- standard* 100

REFERENCE VALUE:

Plasma Fasting Glucose: 70- 100mg/dL

QUANTITATIVE ESTIMATION OF HbA_{1C}

METHOD

Particle enhanced Immunoturbidimetric method

PRINCIPLE

Total haemoglobin and HbA_{1C} are combining in hemolysed blood with equal affinity for particles in R1. The concentrations of both the substances in blood is proportional to the level of binding

Mouse anti-humanHbA_{1C} monoclonal antibody (R1) binds with particle bound HbA_{1C}

Then Goat anti-mouse IgG polyclonal antibody (R3) interacts with R2 and agglutination takes place. The calculated absorbance is proportional to the HbA_{1C} bound particles. This is used to calculate the % of HbA_{1C}

REAGENTS

R1: Buffer- 20mmol/L Latex: 0.14%

R2: Buffer- 10mmol/L and mouse antihuman HbA_{1C} monoclonal antibody: 5.5mg/dL

R3: Buffer- 10mmol/L and goat anti-mouse IgG polyclonal antibody: 67mg/dL

PROCEDURE

Take 20µL of sample and 750µL of reagent (R1), mixed well and incubated for 2 minutes. Then 250µL of reagent (R2) was added and mixed, incubated for 3 minutes and then 125µL of reagent (R3) was added and mixed. After 2 minutes of incubation absorbance was measured at 660nm.

Temperature: 31°C

Optical path-length: 1cm

Wavelength: 660nm

CALCULATION:

Concentration of HbA_{1C} in an unknown sample is derived from a calibration curve of appropriate mathematical models.

Limit of detection: 10mmol/mol

Reference range: Non- Diabetic: 20-42mmol/mol or 4-6%

ADA range for Type 2 Diabetes

- NON DIABETIC: 4.5- 6.5%
- PRE DIABETIC: 5.7- 6.4
- DIABETIC: >6.5%

QUANTITATIVE ESTOMATION OF TOTAL CHOLESTEROL

METHOD: Cholesterol Oxidase/ Peroxidase (CHOD- POD) method

PRINCIPLE: Hydrolysis of cholesterol esters leads to cholesterol. Then, photometric estimation of cholesterol is done by coupling reactions with free cholesterol.

The following are the reactions

Cholesterol ester + H_2O ⟶ Cholesterol + Fatty acid

Cholesterol + ½O_2 + H_2O ⟶ Cholestenone + H_2O_2

$2H_2O_2$ + 4-Aminoantipyrene + Phenol $\xrightarrow{\text{Peroxidase}}$ Quinoneimine + $4H_2O$

The intensity of the color is proportional the concentration of Cholesterol in the serum

REAGENTS

Sodium Cholate	0.5mmol/L
Phenol	28mmol/L
Pipes	35mmol/L
Peroxidase	>0.8U
Cholesterol oxidase	>0.1U/mL
4-Aminoantipyrene	0.5mmol/L
Cholesterol esterase	>0.2U/mL
Ph	7.0
Standard	5ml; cholesterol- 200mg/dL

STORAGE: 2-8

PREPARATION OF WORKING SOLUTION

Ready to use reagents and standards are allowed to attain room temperature

PROCEDURE

Prior to use, the sample and the working solution should be brought to room temperature

SYSTEM PARAMETERS

Reaction type:	End- point assay
Reaction slope:	Increasing
Flow cell Temperature:	room temperature/ 27°C
Sample volume:	10µL
Reagent volume:	1mL
Delay time:	5 sec
Wave length:	500± 10nm
Optical path-length:	10mm
Standard concentration:	200mg/dL
Zero setting:	With distilled water

PROCEDURE

Three test tubes are labelled as blank (B), test (T) and standard (S). one mL of working reagent is added to all the 3 test tubes. 10µL of standard is added to test tube (S) and 10µL of standard is added to test tube S and 10µL of

the sample is added to test tube “T”. Then it is mixed, incubated for 10 minutes at room temperature.

REAGENTS	BLANK	STANDARD	TEST
Distilled water	10µL	--	--
Reagent	1mL	1mL	1Ml
Standard	--	10µL	--
Sample	--	--	10Ml

CALCULATION

$$\frac{\text{Sample Absorbance}}{\text{Standard absorbance}} \times 200 = \text{concentration of sample (mg/dL)}$$

LINEARITY

Linearity is upto 1000mg/dL

REFERENCE VALUE

Serum total cholesterol

Desirable : <200mg/dL

Borderline : 200- 239mg/dL

High : >239mg/dL

QUANTITATIVE ESTIMATION OF SERUM TRIGLYCERIDES

METHOD:

Colorimetric enzymatic test using Glycerol-3-phosphate oxidase (GPO)

PRINCIPLE

Estimation is based on the enzymatic splitting with lipoprotein lipase. Quinoneimine is an indicator, generated from 4-aminoantipyrine and 4-chlorophenol by hydrogen peroxide under catalytic action of peroxidase.

The reaction sequences are as follows:

$$\text{Triglycerides} \xrightarrow{\text{LIPOPROTEIN LIPASE}} \text{Glycerol + Fatty Acid}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{GLYCEROL KINASE}} \text{Glycerol-3-phosphate + ADP}$$

$$\text{Glycerol-3-phosphate + } O_2 \xrightarrow{\text{GLYCEROL-3-PHOSPHATE OXIDASE}} \text{Dihydroxyacetone phosphate + } H_2O_2$$

$$2H_2O_2 \text{ + Aminoantipyrene + Chlorophenol} \xrightarrow{\text{PEROXIDA}} \text{Quinoneimine + HCl + } 4H_2O$$

REAGENTS

4-Chlorophenol	4mmol/L
Mg^{2+}	15mmol/L
ATP	2mmol/L
4-Aminoantipyrene	0.5mmol/L
Glycerolkinase	≥0.4Ku/L
Peroxidase	≥2kU/L
Lipoprotein lipase	≥2kU/L
Good's buffer	pH 7.2; 50mmol/L
Glycerol-3-phosphate Oxidase	≥0.5kU/L

STANDARD Triglyceride 200mg/dL

STORAGE at 2- 8°C

PROCEDURE

The sample and the working solution are brought to room temperature

Three test tubes are taken and labelled as standard (S), test (T) and blank (B). one mL of working reagent is added to three test tubes. 10µL of sample is added to test tube labelled test (T). 10µL of standard is added to the tube labelled S. Then mixed and incubated for 10 minutes at room temperature.

REAGENT	BLANK	STANDARD	TEST
Distilled water	10Ml	--	--
Reagent	1Ml	1mL	1Ml
Standard	--	10µL	--
Sample	--	--	10µL

CALCULATION

$$\frac{\text{Sample absorbance}}{\text{Standard absorbance}} = \text{Concentration of sample (mg/dL)}$$

Subtracting 10mg/dL from Triglycerides value for free glycerol correction.

LINEARITY

From 2-1000mg/dL

GENERAL SYSTEM PARAMETERS

Reaction type	End-point
Wavelength	500nm
Reaction slope	Increasing
Delay time	5 seconds
Flow cell temperature	Room temperature/ 27°C
Reagent volume	1.0mL
Sample volume	10µL
Std concentration	200mg/dL
Optical path	1cm
Zero-setting	with distilled water

The instrument is set with the above parameters.

REFERENCE VALUES

Serum triglycerides

Normal	<150mg/dL
High	150- 199mg/dL
Hypertriglyceridemi	200- 499mg/dL
Very high	>499mg/dL

QUANTITATIVE ESMITATION OF SERUM HIGH DENSITY LIPOPROTEIN CHOLESTEROL

METHOD Direct enzymatic method

PRINCIPLE Antibodies against human lipoproteins are utilized to form antigen-antibodies complexes with chylomicrons, LDL, VLDL, and in a way that only HDL-cholesterol is selectively estimated by an enzymatic cholesterol measurement.

The reaction sequence is as follows

VLDL, LDL, Chylomicrons ⟶ Antigen-antibody complexes + HDL

HDL-Cholesterol + H_2O + O_2 ⟶ Cholest-4-en-3-one + Fatty acid + H_2O_2

H_2O_2 + F-DAOS + 4-Aminoantipyrene $\xrightarrow{\text{PEROXIDASE}}$ Blue complex + H_2O

REAGENTS

Reagent 1:

Good's buffer pH 7.0 25mmol/L

Peroxidase 2000U/L

Ascorbate Oxidase 2250U/L

Anti-human β-Lipoprotein Antibody (sheep)

4-Aminoantipyrene 0.75mmol/L

Reagent 2:

Good's buffer pH 7.0 30mmol/L

Cholesterol Oxidase 20000U/L

Cholesterol esterase 4000U/L

N-ethyl-N-(2-hydroxy-3-sulfopropyl) 0.8mmol/L

Sodium salt (F-DAOS), 3,5-dimethoxy-4-fluoroaniline

CALIBRATOR

HDL-cholesterol 50.6mg/dL

STORAGE 2-8°C

PROCEDURE

The sample and working solution are allowed to attain room temperature prior to use

GENERAL SYSTEM PARAMETERS

Reaction type End-point

Wavelength 600/700nm

Reaction slope increasing

Delay time 5 seconds

Flow-cell temperature Room temperature/ 27°C

Reagent 1 volume 240µL

Reagent 2 volume 60µL

Sample volume 2.4µL

Calibrator 50.6mg/dL

Zero-setting with distilled water

Optical path-length 1cm

The instrument is set with the above system parameters

REAGENT	BLANK	CALIBRATOR	TEST
Calibrator	--	2.4µL	--
Sample	--	--	2.4µL
Distilled water	2.4µL	--	--
Reagent 1	240µL	240µL	240µL

Mixed, incubated at 37°C for 5 minutes; absorbance A_1 was read. Then added

Reagent 2	60Ml	60µL	60µL

Mixed, incubated for 5 minutes at 37°C; absorbance A_2 was measured.

PROCEDURE

Three test tubes were labelled as test (T), blank(B) and Calibrator(C). 240µL of working reagent1 was added to all three test tubes. 2.4µL of sample was added to test (T) and 2.4µL of calibrator was added to C-Tube. Then mixed and incubated for 5 minutes at room temperature. The absorbance A_1 was read, then 60µL of working reagent2 was added to all the three test tubes. It was mixed and incubated for 5 minutes at room temperature. Absorbance A_2 was read.

$$\Delta A = (A_2 - A_1)\text{sample or calibrator}$$

CALCULATION

$$\frac{\Delta A \text{ Sample}}{\Delta A \text{ Calibrator}} \times \text{Calibrator concentration} = \text{Sample concentration (mg/dL)}$$

LINEARITY

This method is used to determine HDL-C concentration within a measuring range from 1- 180mg/dL

REFERENCE RANGE

Serum HDL-C

Low <40mg/dL

Desirable 40- 60mg/dL

FERRIC REDUCING ANTI-OXIDANT POWER ASSAY PROCEDURE

PRINCIPLE

Ferric reducing antioxidant power (FRAP) assay uses antioxidants as reductants in a redox-linked colorimetric reaction. Here Fe^{3+} (Ferric) is reduced to Fe^{2+} (ferrous) at low pH forming a colored ferrous-probe complex from a colorless ferric-probe complex. The color is then read spectrophotometrically at 594nm at $37^{O}C$.

Antioxidants are reducing agents that donate electrons to free radicals to stabilize them and minimize the damage caused by free radicals to DNA, cells and organ systems. Eg. polyphenols; flavonoids; vitamins and enzymes like glutathione peroxidase and superoxide dismutase. This assay can detect antioxidant capacities as low as 0.2 mM Fe^{2+} equivalents.

REAGENT PREPARATION:

3.5ml distilled water is added to Reagent B and mixed thoroughly and refrigerated at -20°C

FRAP working solution is prepared by mixing reagents A, B & C in the ratio of 10:1:1 just before assay.

STANDARD PREPARATION:

Standard should be prepared just before assay

SAMPLE	STANDARD (µL)	DILUENT (µL)	FRAP (µM)
S1 (Blank)	0	1000	0
S2	2.5	997.5	100
S3	5	995	200
S4	7.5	992.5	300
S5	10	990	400
S6	12.5	987.5	500
S7	15	985	600
S8	17.5	982.5	700
S9	20	980	800

ASSAY PROCEDURE

1. 10µL of the sample or standard is added in each well.
2. 220µL of the working solution is added in each well.
3. The solution is mixed by continuous stirring for 4 minutes

4. The mixture is then read at 593nm

DATA ANALYSIS

1. Zeroed absorbance values:

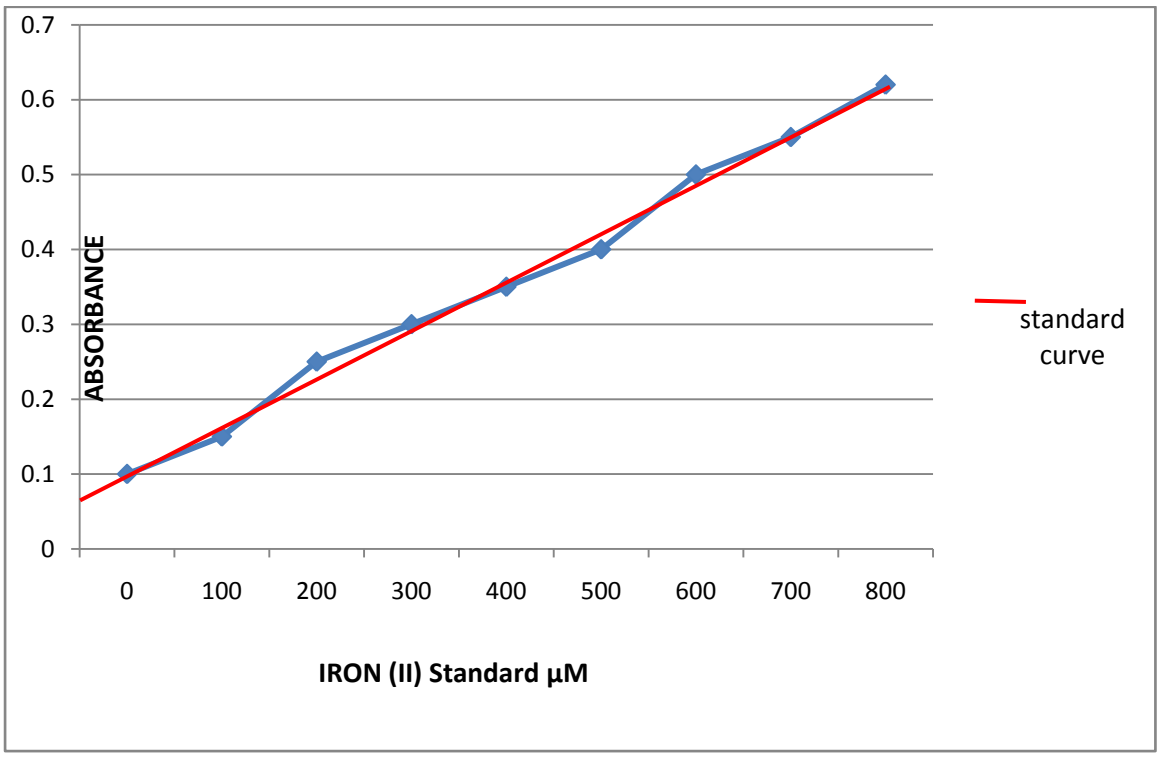
   ΔA593nm = ΔA593nm sample (or) standard - A593nm blank

   Where **A593nm sample (or) standard** is the absorbance measured 4 minutes after the addition of antioxidants from samples or standards.

2. The zeroed absorbance (ΔA593nm) of standards are plotted as a function of their final concentrations in the above table.

3. The FRAP values are calculated using the equation obtained from the linear regression of the standard curve with substituted ΔA593nm values for each sample.

FRAP (μM) = (ΔA593nm– intercept)/ slope



RESULTS AND STATISTICAL ANALYSIS

This study was done to evaluate the metabolic risk factors and total anti-oxidant capacity in Type 2 DM patients. It encompassed a total of 100 subjects with 50 cases of known type 2 DM patients and 50 controls (subjects without type 2 DM).

Master table 1 gives the characteristics of Type 2 DM patients along with their biochemical parameters.

Master table 2 gives the characteristics of control group along with their biochemical parameters.

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS package software

1. Pearson's correlation was used for univariate analysis

2. The groups were compared using Student's t-test

3. P-value of <0.05 is considered significant

TABLE 1

AGE BETWEEN CASES AND CONTROLS

	GROUP	N	MEAN	STD DEVIATION	P-VALUE
AGE	CASES	50	50.61	5.717	0.131
	CONTROL	50	49.02	3.944	

TABLE 2

BMI BETWEEN CASES AND CONTROLS

VARIABLE	GROUP	N	MEAN	STD DEVIATION	P-VALUE
HEIGHT	CASE	50	155.3404	9.05845	0.105
	CONTROLS	50	158.2553	8.19721	
WEIGHT	CASES	50	66.7234	12.22382	0.641
	CONTROLS	50	67.7872	9.71090	
BMI	CASES	50	27.8051	5.39825	0.406
	CONTROLS	50	27.0447	3.14850	

TABLE 3

GROUP Vs GENDER CROSS TABULATION AMONG THE GROUPS

	GROUP		TOTAL
	DM	CONTROL	
FEMALE count	29	28	57
% within group	58%	56%	
% of total	51%	49%	
MALE count	21	22	43
% within group	42%	44%	
% of total	49%	51%	
TOTAL COUNT	50	50	100
% within group	100%	100%	100%
% of total	50%	50%	100%

TABLE 4

COMPARISON OF FASTING BLOOD SUGAR BETWEEN CASES AND CONTROLS

	GROUP	N	MEAN	STD. DEVIATION	P-VALUE
FBS	CASES	50	155.81	61.80	0.00
	CONTROL	50	81.96	11.55	

The mean glucose values of cases is 155.8 mg/dL and that of the controls is 81.96 mg/dL. The p-value is <0.01 and it is statistically significant

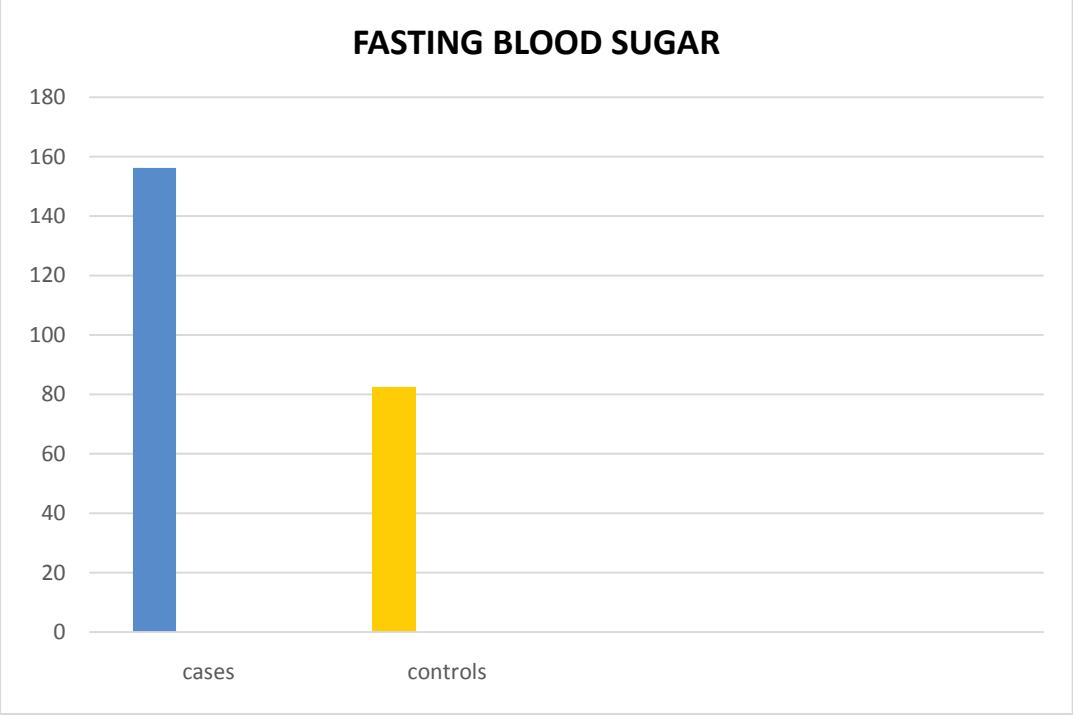


TABLE 5

COMPARISON TOTAL CHOLESTEROL BETWEEN CASES AND CONTROLS

TOTAL CHOLESTEROL	GROUP	N	MEAN	STD DEVIATION	P-VALUE
	CASES	50	241.3404	29.12065	0.000
	CONTROLS	50	155.0851	20.70164	

The mean value of serum total cholesterol in cases is 241.34mg/dL and that of controls is 155.09mg/dL with a p-value <0.01.

TABLE 6

COMPARISON OF SERUM TRIGLYCERIDES BETWEEN CASES AND CONTROLS

	GROUP	N	MEAN	STD. DEVIATION	P-VALUE
TGL	CASES	50	262.89	34.03	0.00
	CONTROLS	50	101.56	21.48	

TABLE 7

COMPARISON OF HDL BETWEEN CASES AND CONTROLS

	GROUP	N	MEAN	STD. DEVIATION	P-VALUE
HDL	CASES	50	22.62	5.27	0.00
	CONTROLS	50	55.77	9.10	

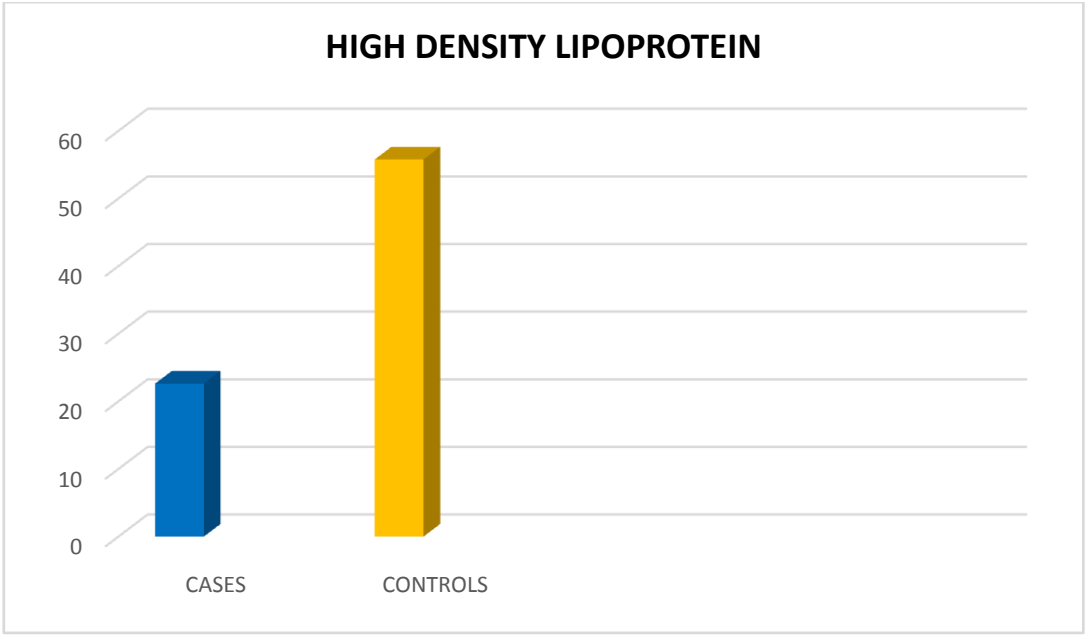


TABLE 8

COMPARISON OF HbA_{1C} BETWEEN CASES AND CONTROLS

	GROUP	N	MEAN	STD DEVIATION	P-VALUE
HbA_{1C}	CASES	50	8.87	2.05	0.000
	CONTROLS	50	5.61	0.33	

TABLE 9

COMPARISON OF PLASMA TOTAL ANTIOXIDANT CAPACITY

BETWEEN CASES AND CONTROLS

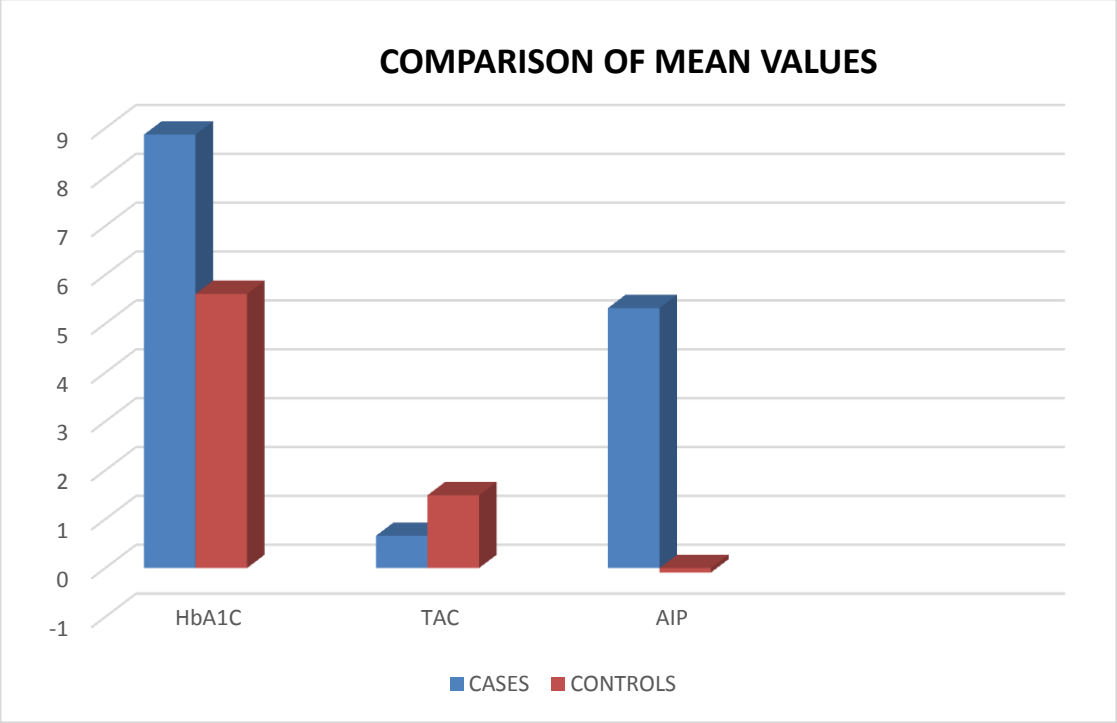
	GROUP	N	MEAN	STD DEVIATION	P-VALUE
TAC	CASES	50	0.65	0.14	0.000
	CONTROLS	50	1.49	0.29	

TABLE 9

COMPARISON OF ATHEROGENIC INDEX OF PLASMA BETWEEN

CASES AND CONTROLS

	GROUPS	N	MEAN	STD DEVIATION	P-VALUE
AIP	CASES	50	-0.09	+0.12	0.000
	CONTROLS	50	+5.32	+1.60	



CORRELATION OF AGE, BMI, FBS, HbA_{1C}, AIP Vs TAC IN CASES

VARIABLES	PEARSON CORRELATION	P-VALUE
AGE Vs TAC	-0.058	0.565
BMI Vs TAC	-0.060	0.553
FBS Vs TAC	-0.619	0.000
HbA_{1C} Vs TAC	-0.679	0.000
AIP Vs TAC	-0.806	0.000

CORRELATION OF AGE, BMI, FBS, $HbA_{1C,}$ TAC Vs AIP

VARIABLES	PEARSON CORRELATION	P-VALUE
AGE Vs AIP	-0.116	0.252
BMI Vs AIP	-0.031	0.762
FBS Vs AIP	-0.675	0.000
HbA_{1C} Vs AIP	-0.788	0.000
TAC Vs AIP	-0.806	0.000

Here p-value <0.01 is considered significant.

DISCUSSION

It has been found from various studies that the major burden in type 2 DM is the cardiovascular risk due to hyperglycemia and dyslipidemia associated with it. This mechanism of complications from hyperglycemia and dyslipidemia is through the generation free radicals and reactive oxygen species.

In our study we compared the levels of oxidative stress and antioxidant capacity in the blood of people with Type 2 Diabetes Mellitus and that of people without diabetes. The main objective of the study was to understand how far the decrease in TAC and the increase in Oxidative Stress is and to assess the associated cardiovascular risk in type 2 DM patients. The diabetics and the controls were matched for age, gender and BMI. The biochemical parameters were also analysed between them. Among the parameters, Fasting Blood Sugar, HbA_{1C}, High Density Lipoprotein, Triglycerides, Total Cholesterol and Total Antioxidant Capacity were statistically significant between the cases and controls.

The mean FBS and HbA_{1C} values among cases were 155.8mg/dL and 8.87%; and among controls were 81.96mg/dL and 5.67% respectively. The lipid profile between the cases and controls also showed a striking difference and the differences were statistically significant. The mean HDL values among cases and controls were 22.62mg/dL and 55.71mg/dL respectively. The mean total cholesterol and triglyceride values among cases were 241.34mg/dL and

262.89mg/dL and among controls were 155.07mg/dL and 101.56mg/dL respectively. This finding is in accordance with the study by who showed that dyslipidemia could be an early finding in type 2 DM before hyperglycemia itself.

The TAC values were very low among Type 2 Diabetes patients and the values were statistically significant. The results were in agreement with previous studies by B. Sai Ravi Kiran et al[176] and by Dr. Kusuma Kumari[177] in south Indian population. The study by B. Sai Ravi Kiran et al included even metabolic syndrome patients and the TAC values were low in them too. The TAC values in that study for cases and controls were 0.62±0.08 and 1.32±0.14 respectively. The TAC values in our study for cases and controls were 0.65±0.14 and 1.49±0.29. Another similar study in African population by FA Ganjifrockwala[178], et al showed TAC values among cases and controls as 0.48±0.37 and 0.60±0.44 respectively. All these studies thus suggest a significant decrease in TAC values in diabetics.

The TAC values negatively correlated with Fasting Blood sugar, HbA_{1C}, Age and AIP levels and all were statistically significant. TAC also correlated negatively with BMI but there was no statistical significance because the BMI was high among the controls too. The oxidative stress associated with hyperglycemia and dyslipidemia of type 2 DM is responsible for the reduced total anti-oxidant capacity.

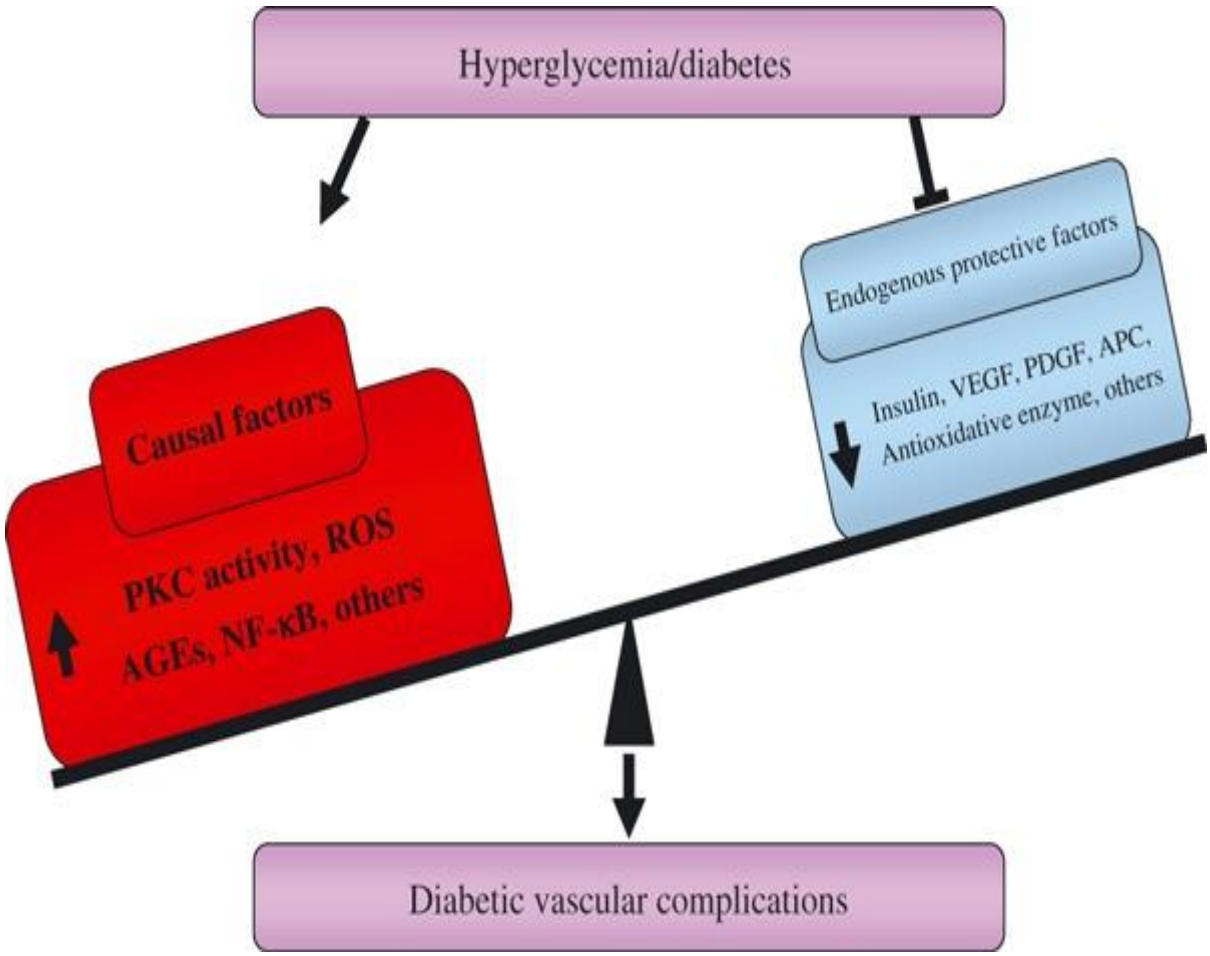
Lipid profile in T2DM correlated positively with HbA1C and FBS values. Diabetic patients especially those with vascular complications show significant alteration in the structure and metabolism of lipids[179]. These evidences suggest lipid profile as a marker of oxidative stress. Though not alone but when combined with other markers it could become a better predictor as a stress biomarker.

FRAP calculates the overall activity of antioxidant enzymes and vitamins. To avoid the toils of measuring each antioxidant component of plasma separately FRAP could be used as a single test to calculate the total antioxidant capacity (TAC) of plasma. Assessing FRAP gives more biologically relevant information regarding the relationship between pro-oxidants and anti-oxidants. FRAP exhibits the status of extra-cellular anti-oxidants that delay the oxidative process. FRAP analysis could be considered as a more relaxed and cost-effective method which could be incorporated into predicting risk for diabetes and CVD.

The protective HDL levels are also very low in these patients that contributed to the high Atherogenic Index of Plasma (AIP) in type 2 diabetes patients. The rise in AIP values among the cases is statistically significant and it followed the pattern observed in studies by Debiasova M and Frohlich J(180). A meta-analysis(181) of fifteen eligible studies with a total population covering 4010 type 2 DM patients also shows high AIP values among diabetics and about 40% patients showed AIP values >4.5.

The study also showed that AIP values predicted risk for Type 2 DM better than many other parameters. The AIP values correlated negatively with FBS, TAC and HbA_{1C} and the correlation was statistically significant. AIP also correlated negatively with age and BMI but the values did not show a statistical significance.

Prevention of cardiovascular and other complications from T2DM should start with screening. Patient selection for screening is not difficult because all the newly diagnosed T2DM patients are considered for screening. Those who were negative for complications during screening can be screened periodically once in a year or once in 6 months depending upon their prognosis. Thus this study could add up to the screening procedure considering the use of TAC measurements.


Hyperglycemia/diabetes
Endogenous protective factors
Insulin, VEGF, PDGF, APC,
Antioxidative enzyme, others
Causal factors
PKC activity, ROS
AGEs, NF-κB, others
Diabetic vascular complications

SUMMARY

This is a case-control study on 50 patients and 50 healthy controls on an urban population done in a tertiary healthcare setup to evaluate the metabolic risk factors and total antioxidant capacity in type 2 Diabetes Mellitus done in the department of Biochemistry in Government Kilpauk Medical College

To assess the metabolic risk factors we have included Fasting and Post-Prandial Blood Sugar, HbA_{1C}, Total Cholesterol, Triglycerides, HDL-c, Atherogenic Index of Plasma.

Total Antioxidant Capacity of plasma is assessed by FRAP method and it is much less time consuming than assessing the capacity of individual antioxidants in blood

From the evaluation it's found that decrease in TAC and increase in metabolic risk factors are the primary causes for complications in type 2 Diabetes Mellitus, especially the increase in lipid profile. TAC used in this study helps in predicting cardiovascular and cerebrovascular risk of T2DM patients. The results obtained were statistically significant as well. Thus this could be considered as a screening procedure for complications in any T2DM patients just after diagnosis.

CONCLUSION

This study examined the relationship between cardiovascular risk and type 2 diabetes through the evaluation of oxidative stress and total antioxidant capacity. It is found that insulin resistance is the culprit of hyperglycemia and dyslipidemia in type 2 DM which in turn is responsible for the increased oxidative stress. This links type 2 diabetics directly into life-threatening complications of atherosclerosis.

All the cases had a very high Atherogenic Index of Plasma and it correlated positively with HbA1C, TAC and FBS. So, it's understood that in type 2 DM, the hyperglycemic status(FBS/PPBS) and its duration(HbA1C) both are involved in the complications of type 2 DM.

The study therefore suggests, the estimation of plasma antioxidants levels with other routine investigations may be useful in the prevention of the diabetic complications. Decreased TAC values could be considered as an early marker in the pathogenesis of complications of T2DM. Astute treatment could improve longevity and quality of life of the patient or can even revert some complications.

LIMITATIONS OF THE STUDY

The following limitations are observed in the study conducted:

- The relatively small sample size doesn't give a very significant result
- The Total Antioxidant Capacity of the plasma analysed by FRAP method has a few limitations including its inability to analyse the activity of glutathione peroxidase, catalase
- The BMI of both the cases and controls were relatively similar and did not show a statistical significance
- The cases included type 2 DM patients with varying duration of disease

SCOPE FOR FURTHER STUDIES

- Population and ethnicity based reference intervals for TAC values is needed
- ROLE OF total antioxidant capacity values in routine blood tests for T2DM Patients
- Modification of TAC methods to make it less expensive
- Role of TAC values in monitoring patients with complications of T2DM viz., retinopathy, nephropathy or cardiovascular events
- Classifying patients according to TAC values for better treatment approaches.

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INSTITUTIONAL ETHICS COMMITTEE
GOVT. KILPAUK MEDICAL COLLEGE,
CHENNAI-10
Protocol ID. No. 02/2017 Meeting held on 14.11.2017

The Institutional Ethical Committee of Govt. Kilpauk Medical College, Chennai reviewed and discussed the application for approval **"EVALUATION OF METABOLIC RISK FACTORS AND TOTAL ANTIOXIDANT CAPACITY IN TYPE 2 DIABETES MELLITUS IN URBAN POPULATION"** submitted by Dr.B.SATHYA , Post Graduate in Bio-Chemistry,Govt. Kilpauk Medical College, Chennai-10.

The Proposal is **APPROVED.**

The Institutional Ethical Committee expects to be informed about the progress of the study any Adverse Drug Reaction Occurring in the Course of the study any change in the protocol and patient information /informed consent and asks to be provided a copy of the final report.

15.11.2017.
DEAN
Govt. Kilpauk Medical College,
Chennai-10.

15-11-17

ME 1 Sec> Ethical Committee

URKUND

Document	EVALUATION OF TOTAL ANTIOXIDANT CAPACITY AND METABOLIC RISK FACTORS IN TYPE 2 DIABETES PATIENTS IN A TERTIARY CENTRE.docx (D41902155)
Submitted	2018-09-28 19:16 (+05:0-30)
Submitted by	B. Sathya (nalamnmc@gmail.com)
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3% of this approx. 24 pages long document consists of text present in 3 sources.

PROFORMA

NAME: OP NO:

AGE/SEX:

ADDRESS: OCCUPATION:

DATE:

PRESENT HISORY: Duration of Diabetes

H/O treatment for diabetes, dyslipedemia

PAST HISTORY: History of cardiac/kidney disease.

History of any arthritis, malignancy

PERSONAL HOISORY:

- H/O Smoking, Alcohol intake.

FAMILY HISTORY:

- H/O Diabetes
- H/O Hypertension

ON EXAMINATION:

GENERAL EXAMINATION:

VITALS:

BP: PULSE RATE:

CVS: RS:

PER ABDOMEN: CNS:

INVESTIGATIONS:

1. Fasting blood sugar
2. Post prandial blood sugar
3. HbA1c
4. triglycerides
5. Total cholesterol
6. HDL
7. VLDL
8. Total Antioxidant Capacity

PATIENT CONSENT FORM

STUDY TITLE: EVALUATION OF METABOLIC FACTORS AND TOTAL ANTIOXIDANT CAPACITY IN TYPE 2 DIABETES MELLITUS IN URBAN POPULATION

STUDY CENTRE:GOVT. KILPAUK MEDICAL COLLEGE HOSPITAL, CHENNAI-10

PATIENT'S NAME:

PATIENT'S AGE: SEX:

IDENTIFICATION NUMBER:

I confirm that I have understood the purpose and procedure of the above study. I have the opportunity to ask any questions and all my questions and doubts have been answered to my complete satisfaction.

I understand that my participation in this study is voluntary and that I am free to withdraw at any time without giving reason, without my legal rights being affected.

I understand that the sponsor of clinical study, working on sponsor's behalf, the ethical committee and the regulatory authorities will not need my permission to look at my health records, both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the study I agree to this access. However I understand that my identity would not be revealed in any information released to third parties unless as required under the law. I agree not to restrict the use of any data or results that arise from the study.

I hereby consent to participate in this study.

I hereby give permission to undergo complete clinical examination and diagnostic tests including hematological, biochemical and radiological tests.

Signature/thumb impression Signature of the investigator:

Patient's name and address: Name of the investigator:

Place:

Date:

CONTROLS

S.NO	NAME	AGE	SEX	HT	WT	BMI	FBS	T.CHL	TGL	HDL	HbA1C	TAC	AIP
1	Gunalan	47	M	160	84	32.81	99	156	88	45	6	1.22	-0.07
2	Deviga	48	F	155	70	29.13	97	177	93	60	6	1.67	-0.17
3	Kabir Ahmed	48	M	165	81	29.75	99	183	76	52	5.5	1.34	-0.19
4	Virudachalam	59	M	159	79	31.24	98	145	105	56	5.8	1.40	-0.08
5	Mahalakshmi	47	F	150	59	26.22	78	167	78	67	5.6	1.56	-0.29
6	Gokila	48	F	140	60	30.61	81	173	115	62	5.5	1.12	-0.09
7	Suryakumari	47	F	142	54	26.78	84	155	87	56	5.9	1.56	0.17
8	Valli	45	F	150	55	24.44	81	170	144	49	5.8	1.09	+0.11
9	Kandasamy	48	M	170	75	25.95	80	143	98	47	5.3	1.70	-0.04
10	Veerannan	49	M	151	71	31.13	85	157	78	50	5.4	1.68	-0.17
11	Poornima	46	F	155	65	27.05	97	138	96	58	5.3	1.35	-0.14
12	Uma	46	F	150	81	36	89	176	132	63	5.4	1.89	-0.04
13	Yeshoda	47	F	155	70	29.13	92	154	103	65	5.7	1.44	-0.16
14	Baskar	45	M	165	56	20.56	87	178	89	52	5.9	1.67	-0.12
15	Saraswathi	50	F	150	50	22.22	89	134	145	70	5.9	1.39	-0.04
16	Mullai	42	F	160	73	28.51	85	197	77	69	5.9	1.39	-0.31
17	Dandapani	48	M	170	72	24.91	75	133	121	48	5	1.44	+0.04
18	Sudha	49	F	163	74	27.85	63	128	88	72	5.7	1.34	-0.28
19	Saveetha	47	F	155	54	22.47	59	168	92	68	6	1.59	-0.23
20	Kalai	48	F	150	62	27.55	75	164	76	67	6.0	1.38	-0.30
21	Gnyanam	56	M	155	60	24.97	71	142	94	46	5.7	1.51	-0.05
22	Selvaganapathy	56	M	173	62	20.71	65	156	135	54	5.7	1.32	+0.04
23	Roja	48	F	147	58	26.84	60	185	144	52	5.5	1.45	+0.09
24	Lakshmiammal	45	F	154	60	25.29	81	157	124	54	5.9	1.49	0.00
25	Nasreen	56	F	159	67	26.5	95	149	132	48	5.0	1.82	+0.08
26	Pichai	55	F	155	59	24.55	84	135	113	69	5.8	1.56	-0.14
27	Ambika	46	F	158	67	26.83	85	164	92	58	5.6	1.71	-0.16
28	Pounammal	49	F	163	60	22.58	87	126	68	49	5.6	1.43	-0.21
29	Kanaga	46	F	164	78	29.0	99	184	87	66	6.0	1.33	-0.29
30	Vijaya	53	F	158	72	28.84	99	178	132	59	5.8	1.25	0.00

31	Shanthi	52	F	157	75	30.42	84	122	125	54	6.0	1.45	0.00
32	Revathy	46	F	160	69	26.95	90	146	99	48	5.5	1.14	-0.05
33	Subramani	48	M	170	81	28.02	72	177	97	39	5.9	1.54	+0.08
34	Ponnambalam	50	M	163	83	31.23	64	145	79	56	5.2	1.55	-0.21
35	Sivayya	52	M	160	69	26.95	73	156	89	43	5.6	1.06	-0.04
36	Aandal	52	F	157	71	28.8	64	166	132	51	5.7	1.80	+0.05
37	Malliga	50	F	154	63	26.56	60	179	125	55	4.9	2.23	0.00
38	Jaya	47	F	155	59	24.55	85	190	110	49	5.0	1.42	-0.01
39	Kusalambal	49	F	149	60	27.02	85	144	89	54	5.7	1.45	-0.14
40	Kuruvammal	45	F	165	73	26.81	73	109	96	80	5.8	2.33	-0.28
41	Senthil	46	M	153	70	29.90	95	132	85	58	5.8	1.36	-0.19
42	Sekar	51	M	168	79	27.99	76	155	101	45	5.9	1.27	-0.01
43	Chinnathai	45	F	159	62	24.52	84	128	78	67	5.1	2.39	-0.29
44	Kumaran	60	M	159	53	20.96	75	134	76	47	4.8	1.22	-0.15
45	Poovarasan	57	M	153	60	25.64	89	124	80	50	5.6	1.05	-0.15
46	Velayutham	59	M	178	89	28.16	90	145	93	45	6.0	1.56	-0.18
47	Narendiran	45	M	177	82	26.20	74	165	115	49	5.2	1.32	+0.01
48	Suguna	49	F	148	60	27,40	77	160	134	56	5.4	1.76	+0.02
49	Poongodi	54	F	150	68	30.22	75	162	122	53	4.9	1.23	0.00
50	Muthu	56	M	156	68	26.56	87	144	101	62	5.4	1.34	-0.15

CASES

S. NO	NAME	AGE	SEX	HT	WT	BMI	FBS	T. CHL	TGL	HDL	HbA1C	TAC	AIP
1	Govindan	47	M	162	48	18.28	210	202	254	23	7.1	0.72	4.86
2	Selvan	48	M	175	65	21.22	180	192	300	25	10.8	0.65	5.22
3	Raman	48	M	150	50	22.22	122	251	256	26	9.4	0.58	4.31
4	Darani	59	F	145	72	34.24	185	255	309	27	9.9	0.87	4.99
5	Ragu	47	M	163	56	21.07	94	213	298	21	6.7	0.59	6.24
6	Ravi	48	M	159	47	18.59	174	255	309	20	8.5	0.77	6.71
7	MohdMubin	47	M	142	58	28.76	80	196	288	21	8.2	0.83	6.02
8	Sivanesan	45	M	146	67	31.43	141	201	260	21	8.6	0.67	5.44
9	Venda	48	F	162	59	22.48	123	253	278	23	8.1	0.63	5.32
10	Rooban	49	M	155	60	24.97	106	243	221	25	8.8	0.57	3.85
11	Prema	46	F	156	68	27.94	336	267	295	22	12.9	0.66	5.84
12	Sheila	46	F	160	71	27.73	95	226	293	23	7.2	0.65	5.61
13	Appannan	47	M	172	88	29.74	134	235	283	19	7.3	0.72	6.53
14	Perumal	45	M	163	71	26.72	165	265	272	13	11.5	0.68	9.03
15	Athira	50	F	154	59	24.87	152	259	256	23	9.0	0.93	4.90
16	Kannabiran	42	M	145	67	31.86	283	297	232	10	10.0	0.58	10.08
17	Sumathi	48	F	159	70	27.68	191	266	265	15	12.2	0.94	7.67
18	Rajalakshmi	49	F	164	60	22.3	337	250	259	18	9.3	0.39	6.35
19	Padma	47	F	160	48	18.75	173	243	303	22	9.6	0.52	6.00
20	Ananda Pandian	48	M	150	67	29.77	253	196	257	24	13.1	0.48	4.68
21	Gopi	56	M	156	67	27.53	92	198	244	20	7.9	0.55	5.31
22	Selvi	56	F	153	57	24.34	280	205	191	26	11.2	0.57	3.22
23	Mohan	48	M	156	55	22.60	142	236	250	17	7.3	0.63	6.41
24	Arul	45	M	162	60	22.86	62	273	278	30	7.2	0.62	4.03
25	Noor Jahan	56	F	162	67	25.52	100	278	300	25	7.6	0.67	5.06
26	Valliammal	55	F	140	60	30.61	114	223	309	26	7.2	0.66	5.21
27	Nesan	46	M	145	59	28.06	166	209	278	27	8.1	0.58	4.49
28	Karuppan	49	M	147	62	28.69	131	221	255	33	7.4	0.87	3.39
29	Mary	46	F	140	59	30.10	162	289	239	23	8.0	0.85	4.58
30	Poongothai	53	F	155	95	39.54	101	236	223	26	9.9	0.63	3..76

31	Pachaiyammal	52	F	150	75	33.33	181	244	245	19	8.5	0.62	5.65
32	Asogan	46	M	156	78	32.05	108	254	224	18	10.3	0.65	5.38
33	Rani	48	F	145	59	28.06	223	265	276	19	9.2	0.49	6.37
34	Vasantha	50	F	150	74	32.88	164	223	264	23	7.9	0.54	5.05
35	Narayani	52	F	145	78	37.09	151	287	263	22	7.4	0.59	5.21
36	Kodai	52	F	159	60	23.73	148	223	198	28	8.0	0.62	3.11
37	Renuka	50	F	150	64	28.44	121	256	281	27	8.5	0.60	4.61
38	Babu	47	M	170	75	25.95	167	233	296	22	13.5	0.70	5.86
39	Shankar	49	M	172	84	28.39	142	243	244	15	8.1	0.97	7.08
40	Nachiyal	45	F	149	75	33.78	188	234	287	19	7.5	0.54	6.61
41	Balaraman	46	M	155	59	24.55	143	221	234	11	15.7	0.66	9.43
42	Bhuvaneshvari	51	F	150	95	42.22	132	292	247	23	7.3	0.75	4.73
43	Krishnan	58	M	178	89	28.08	154	289	196	38	7.8	0.39	2.26
44	Rukmani	45	F	155	95	39.54	116	187	189	25	7.0	0.62	3.29
45	Ganga	60	F	160	71	27.73	106	254	228	28	7.2	0.66	3.58
46	Menaka	60	F	150	56	24.88	119	262	298	23	6.4	0.91	5.71
47	Jayaseeli	53	F	149	57	25.67	76	243	331	29	6.4	0.44	4.99
48	Aruna	48	F	152	60	25.97	168	230	192	38	9.1	0.88	2.21
49	Varalakshmi	45	F	145	58	27.62	178	221	278	29	10.1	0.64	4.19
50	Gomathi	54	F	150	59	26.22	166	276	306	33	9.5	0.43	4.07